Université de Montréal

Localisation d'un locus pour trait quantitif pour l'hypertension sur le chromosome 2 du rat Dahl.

Présenté par

Vasiliki Eliopoulos
[redacted]

Programme de biologie moléculaire
Faculté des études supérieures

Mémoire présenté à la Faculté des études supérieures
En vue de l'obtention du grade de Maître ès sciences (M.Sc.)

Mars 2006

Université de Montréal
Faculté des études supérieures

Ce mémoire intitulé

Localisation d'un locus pour trait quantitif pour l'hypertension sur le chromosome 2 du rat Dahl.

Présenté par

Vasiliki Eliopoulos

a été évalué par un jury composé des personnes suivantes :

Isabelle Royal
président-rapporteur

Alan Y. Deng
Directeur de Recherche

John White
Membre de jury

Résumé français

L'hypertension essentielle constitue un facteur de risque important pour les maladies cardiovasculaires et affecte aussi un grand pourcentage de la population humaine. La pression artérielle (PA) chez les animaux est influencée à la fois par des facteurs génétiques et environnementaux. L'hypertension est définie en génétique comme étant un trait quantitatif et multifactoriel sous contrôle polygénique. Nos études antérieures par analyse de liaison ont suggéré la présence d'un locus pour trait quantitatif (QTL) associé à la pression artérielle sur le chromosome 2. Notre objectif était de vérifier ces résultats à l'aide de modèles animaux congéniques.

Afin d'étudier l'hypertension dans un environnement homogène, nous utilisons des souches de rats co-sanguines, où tous les animaux sont génétiquement identiques. Des lignées congéniques sont construites par une série de croisements entre le rat S (Dahl *salt-sensitive* hypertendu) et le rat L (rat Lewis normotendu). Ceci résulte en une souche congénique dont le génome provient de la souche hypertendue S, sauf pour une région qui est remplacée par la région homologue de la souche normotendue Lewis. Ces lignées permettent ainsi d'associer un changement potentiel de la PA sur le chromosome 2 lorsque comparée à la lignée S.

Nous avons obtenu des résultats pour 4 souches congéniques. Deux de ces quatre souches ont montré la même pression artérielle moyenne (MAP) que le rat S. Donc, elles ne sont pas significativement différentes par rapport à S. La souche congénique C2S.L1 pourrait être classifiée comme une souche « hyper » hypertendue de S dû au fait que sa préssion artérielle était plus élevée que celle de S même si elle contient des allèles provenant de la souche normotendu Lewis. Les pressions artérielles systoliques et diastoliques varient de façon concordante avec les valeurs de MAP.

Donc, le chromosome 2 du rat Dahl contient un QTL pour la pression artérielle. La découverte de gènes pour ce QTL sur le chromosome 2 peut nous donner une idée des mécanismes qui contrôlent la pathogenèse de l'hypertension.

Mots clefs
Hypertension essentielle, pression artérielle, souche congénique, chromosome 2, rat Dahl *salt-sensitive*, modèle animal rat

Résumé anglais

Essential hypertension is an important risk factor for many cardiovascular diseases and affects a large percentage of the human population. Blood pressure (BP) in animals is influenced at the same time by environmental and genetic factors. In the study of genetics, hypertension is defined as being a multifactorial quantitative trait under polygenic control. Our previous studies using linkage analysis have suggested the presence of a quantitative trait locus (QTL) associated to blood pressure on chromome 2. Our objective was to verify these results using congenic animal models.

In order to study hypertension in a homogeneous environment, we used inbred rat strains where all animals are genetically identical. Congenic strains were obtained by crossing the S rat (Dahl *salt-sensitive* hypertensive) and the L rat (Lewis normotensive rat). They are crossed until we obtained a genetic background of the S rat and the portion containing the QTL replaced by the homologous portion of the L rat. These strains will therefore permit to associate a potential change in arterial pressure on chromosome 2 when compared to the S strain.

We obtained results for four congenic strains. Two of the four strains demonstrated the same mean arterial pressure (MAP) as the S rat. Therefore, they are not significantly different to S. The C2S.L1 strain could be classified as a hyper-hypertensive strain of S due to the fact that its MAP was higher than that of the S strain even thought it contains alleles from the Lewis rat. The diastolic and systolic arterial pressures varied in the same manner as the MAP.

In conclusion, the Dahl rat chromosomes 2 contains a QTL for blood pressure. The discovery of genes for this QTL on rat chromosome 2 could help us to better understand the genetics of hypertension.

Key words
Essential hypertension, blood pressure, congenic strain, chromosome 2, Dahl *salt-sensitive* rat, rat animal model

TABLE OF CONTENTS

LIST OF FIGURES

Figures in article

LIST OF TABLES

List of abbreviations

ACE	Angiotensin converting enzyme
AME	Apparent mineralocortecoid excess
Ang	Angiotensin
ATP	Adenosine triphopshate
BAC	Bacterial Artificial Chromosome
BN	Brown Norway
BP	blood pressure
Ca2+	calcium ion
Chr	chromosome
CHUM	Centre Hospitalier de l'Université de Montréal
cM	centimorgan
CNS	Central Nervous System
Cpb	Capping protein beta
cR	centirad
DAP	Diastolic arterial pressure
EST	Expressed Sequence Tag
Hsp70	Heatshock protein 70
K+	potassium ion
kDa	kiloDalton
kb	kilobase
kg	kilogram
LEW	Lewis Rat
MAP	Mean arterial pressure
Mb	Megabase pairs
mg	milligram
mmHg	millimeter of mercury
MNS	Milan Normotensive
MR	Mineralocorticoid receptor
Na+	sodium ion
NCBI	National Centre for Biotechnology Information
NHANES	Third National Health and Nutrition Examination Survey
bp	base pair
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Locus or Loci
R	Dahl Salt-Resistant rat
RFLP	Restricted Fragmetn Length Polymorphism
RH	Radiation Hybrid

S	Dahl Salt-Sensitive rat
SAP	Systolic Arterial Pressure
sec.	second
SHR	Spontaneously hypertensive rat
SNP	Single Nucleotide Polymorphism
SSLP	Simple Sequence Length Polymorphism
TNFα	Tumor necrosis factor α
VNTR	Variable Number Tandem Repeat
WKY	Wistar-Kyoto rat

Dedications

I dedicate this thesis to the memory of my mother who always pushed me to be a better person and gave me strength to believe in myself and my dreams…

I thank my father for his love and support.

I would also like to thank the rest of my family for being so proud and supportive of my long journey.

A big thanks to my best friend Ana who is my mentor and never gives up on me.

To the girls at the lab: Annie, Julie, Julie, Raphaëlle, Sophie and Chenda. You are truly the definition of teamwork and friendship and I feel privileged to have worked by your sides.

To Dr. Deng who never gave up on me regardless of hard times and showed me true passion for genetics.

To my brother Jimmy who was always there for me,
lifted my spirits and always made laugh.

And last but not least, to my love George, without your love and support this could have never been accomplished, you too inspire me to believe in my dreams…

Introduction

Chapter 1

The concept of hypertension

1.1 High Blood Pressure

Hypertension is a disease that causes organ damage throughout the body due to high blood pressure that is present not only during physical activity but also at rest. The term "high blood pressure" adequately describes a temporary condition caused by stress and physical activity, whereas "hypertension" is a more suitable term for a persistent disease state(1). Diagnosis of essential hypertension includes exclusion of secondary hypertension such as that associated with endocrinological disease, renal disease and renovascular disease (2). A few monogenic diseases (inheritance hypertension) have been defined as unusual hypertension.

Although evidence strongly suggests that essential hypertension is a multifactorial inheritance disease (3), a clear causal gene of essential hypertension has not yet been identified. There are many environmental factors that affect essential hypertension including obesity, diabetes, drinking and smoking. Accounting for these effects is one of the issues that complicate isolation of susceptibility genes.

Blood pressure is the hydrostatic force that the blood exerts against the vessel walls, thus creating a cardiac cycle. Each cardiac cycle is comprised of two alternate phases, the diastolic and the systolic phase. During the systolic phase, the cardiac muscle contracts which in turn creates a propulsion of blood from the cavities of the heart. This in turn supplies the organs of the body with the proper nutrients and essential oxygen. With every contraction of the heart, 70 ml of blood is propelled into the arterial systemic system; this is called the systolic volume. During the diastolic phase, the ventricles fill with blood which is ready to be pumped throughout the body once again. Both the systolic and the diastolic phases have an approximate duration of 0.4 seconds each and induce a pulse of about 65 to 80 beats per minute.

Blood pressure is greater in arteries than in veins and reaches a peak in the arteries during the systolic phase while the heart is contracting; this is called the arterial systolic pressure. Variations of blood pressure depend on cardiac output and the degree of peripheral resistance to the flow of out coming blood. If the volume of blood is increased and the vascular resistance is also increased this in turn augments the pressure.

In medical terms, blood pressure is measured in two parts: systolic and diastolic. The systolic pressure corresponds to the pressure exerted by the blood on the walls right after the passing of blood. A result of typical blood pressure would be presented as such systolic pressure/ diastolic pressure, using as unit millimeters of mercury (mmHg). Optimal pressure being situated around 120/80 mmHg.

The kidneys play a key role if not essential role in the control of blood pressure. There lies a crucial balance between salt intake and peripheral resistance. For example, if the amount of salt intake increases it will be followed by an increase of the extracellular volume and plasmatic volume. This increase in volumes results in escalation of the blood pressure which in turn increases the systolic volume. In the long run, the final effect of the auto regulating systems is increase of the peripheral resistance which brings the systolic volume back to homeostasis. The augmentation of the peripheral resistance could be substantial, this could lead to hypertension. Normal kidneys would minimize these rises in the extracellular fluid and the systolic volume, therefore minimizing the elevations in blood pressure.

In the arterial walls of the body, we also find smooth muscles. The contractions of these smooth muscles contract the arterioles which in turn increase the resistance and consequently increase the arterial pressure. Once the smooth muscles release their pressure, the arterioles dilate and the arterial pressure drops. The muscles in the arterioles obey the signals given to them by nerves, hormones and other messengers.

Physical or emotional stress can also elevate blood pressure by causing nerve end hormonal reactions which will compress the blood vessels. Many other mechanisms also control blood pressure. Baroreceptors which are situated in the blood vessels detect large changes in blood pressure and send the information to the brain to be processed. The factors produced by the blood vessels influence the arterial rigidity and cause vasodilation or a vasoconstriction. Furthermore, atrial natriuretic peptides produced by the brain and the heart, in response to an elevation in pressure in these organs also oppose the vasoconstrictive effect of angiotensin and endothelin and the reabsorption of sodium which is started by the rennin-angiotensin system. These peptides also influence the re-absorption of sodium which is induced by the renal angiotensin system (4).

1.1.1 Renin-angiotensin aldosterone system (RAA)

The RAA (figure1) is vital to the preservation of arterial pressure, homeostasis of the vascular volume and the balance of electrolytes, specifically during the loss of fluids or a drop in pressure. Renin was first isolated in 1898 from rabbit kidneys and was described as a substance that influenced blood pressure (5). This enzyme is released in the circulation by the kidney under the control of multiple signals and acts on angiotensinogen in the liver. Once angiotensinogen is cleaved by the renin protease, angiotensinogen I (Ang I) is created. Ang I is then converted to angiotensin II (Ang II) by the angiotensin conversion enzyme (ACE). Ang II is the principal vasoactive hormone of the RAA system. Its effects include vasoconstriction, the stimulation of aldosterone secretion and the re-absorption of sodium by the kidney. The RAA system acts in the circulation but also exists in many tissues and organs.

The action of Ang II is effective via it's linking to specific receptors (AT1 and AT2). These receptors are part of a family of receptors coupled to G1 proteins and are expressed in many tissues but primarily in the brain, kidney, and the suprarenal glands. Two sub-types of the AT1, AT1α and AT1β receptors were identified in rodents (6).

These two receptors are the product of two genes (Agtr1α and Agtr1β) and are differently expressed and regulated. It was demonstrated that the AT1α receptor was mostly implicated in the regulation of the peripheral vascular rigidity and the response to increased pressure in the central nervous system (SNC). The AT1β receptor is required for the agonistic response of AngII in the SNC (7).

The RAA system works along with other systems to monitor and maintain pressure and fluids. One such system is the sympathetic nervous system. It constitutes a balance to the vasodilation systems. In the majority of patients which suffer from essential hypertension, the arterial pressure drops when treated with ACE inhibitors or antagonists of the AngII receptor. There is also a drop in arterial pressure with certain treatments that activate the RAA system. Therefore one can understand that although it's clear that the RAA system does play a primordial role in controlling blood pressure, it does not constitute the primary cause of hypertension nor is it the only determinant of blood pressure in essential hypertension (8).

All systems controlling or regulating blood pressure act together in a complex fashion. The role of these systems has been established in short term homeostatic responses. It's difficult to examine whether these pathways contribute to blood pressure determination in the long term. Blood pressure must be measured in intact living organisms where all these systems interact in a complex manner, thus making it very difficult to distinguish primary alterations versus adaptive secondary responses.

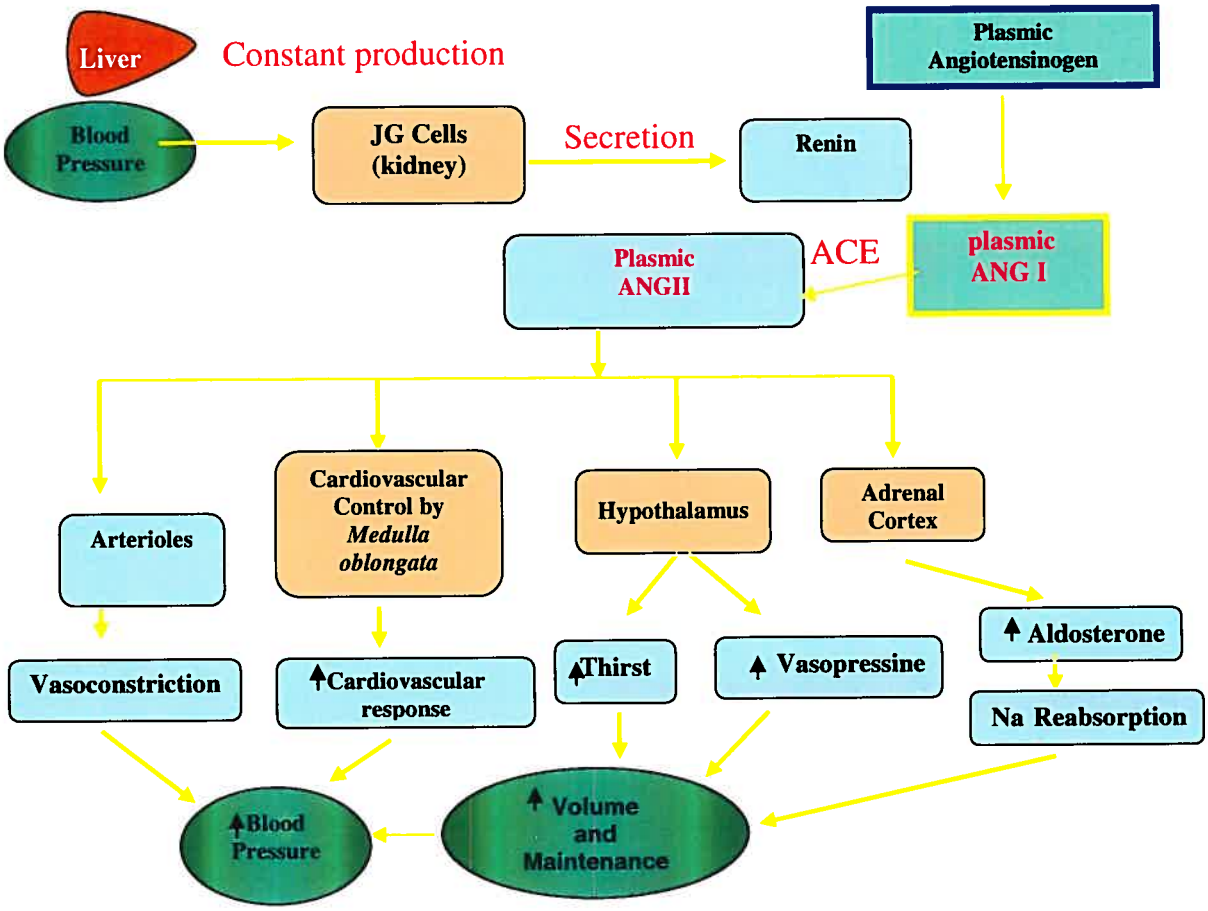


Figure 1. The renin angiotensin system (RAA)

This figure is a schematic representation of the control of arterial pressure on various components. This figure is based on a figure taken from Human Physiology figure 20-13 (5).

1.2 Hypertension

Hypertension (elevated blood pressure levels exceeding 140/90 mmHg according to WHO criteria) is a common complex disorder which affects 15-20% of adult population in Western societies (8). It is classified as primary (essential) or secondary hypertension. The former type is used to describe hypertension without a known pathology. The diagnosis of essential hypertension is made when no other cause for increased blood pressure is found. This form of disease contributes about 90-95% of all hypertension cases. Whereas in 5% of cases, the cause of hypertension is known to be secondary to conditions such as pheochromocytoma, primary hyperaldosteronism (Conn's syndrome), Cushing's syndrome (excessive glucocorticoids), renal disease or drug induced. This disease is considered to be a multifactorial disorder with many genetic, environmental and demographic factors contributing to blood pressure variation. The complex nature of the involved mechanisms makes it difficult to identify a single pathological system of prime importance in regulating blood pressure.

Hypertension is one of the most important risk factors for cardiovascular diseases. It is one of the principal independent risk factors for stroke, myocardial infarction, and end-stage renal disease. Furthermore, it is associated with many other complications that affect vital body functions such as left ventricular hypertrophy, diastolic dysfunction, congestive heart failure, cerebral thrombosis, encephalopathy and retinopathy (9).

1.2.1 Epidemiology

As mentioned previously hypertension is a disease which is very present in our society. It affects about 50% of the population aged 65 and over (10). Also, a 55 year-old with normal blood pressure has a 90% chance of developing hypertension during his or her lifetime. According to the NHANES (Third National Health and Nutrition Examination Survey) only 53.6% of people suffering from hypertension are treated. Surprisingly, only 27.4% of those treated are treated successfully (blood pressure under 140/90mmHg) . It is also clear from family and epidemiological studies that hypertension arises from a complex interplay between genetic and environmental lifestyle exposures including dietary sodium intake, excess alcohol consumption and body weight (10).

1.2.2 Monogenic Hypertension (Mendelian)

Monogenic hypertension is responsible for approximately 5% of hypertension cases and is considered as a unique trait because it demonstrates mendelian transmission. A single defective gene is responsible of the disease and its great variations in blood pressure. A lot of information obtained in the field of hypertension came from single gene disorders from which gene variants causing the trait were characterized (11).

Liddle's syndrome

Liddle's syndrome is an autosomal dominant disorder that leads to increased re-absorption of sodium and water in the renal collecting tubules and hence leads to hypertension. The syndrome was found to be due to mutations in the genes coding for the β and γ subunits of ENaC (epithelial sodium channel).

Syndrome of apparent mineralocorticoid excess (AME)

AME is an autosomal recessive disorder characterized by an early onset of moderate to severe hypertension. Patients with AME1 have a deficit in 11 β-hydroxylase in their system(12). As a result, 11 β-hydroxylase is under the control of the adrenocorticotropic hormone which will be secreted and lead to an increase in salt and water re-absorption as well as a rise in blood pressure.

Mineralocorticoid receptor (MR) activating mutation

Substitution of leucine for serine at codon 810 (S810L) in the mineralocorticoid receptor causes early onset hypertension that is markedly exacerbated in pregnancy. This mutation results in constitutive MR activity and alters receptor specificity. All steroids, including progesterone, that display antagonists properties when bound to the wild type MR are able to activate the mutant receptor (L810).

Pseudohypoaldosteronism type II

Pseudohypoaldosteonism type II is an autosomal dominant disease characterized by severe hypertension, hyperalkemia and sensitivity to thiazide diuretics (12). This results from altering Na+Cl- and K+ handling. Mutations in two members of the WNK kinase family, WNK 1 and WNK 4, cause the disease. Both genes are highly expressed in the kidney.

Names	Mutations	Hereditary Form
Liddle's syndrome	β ENaC γ ENaC	Autosomal Dominant
AME	11 β-hydroxylase	Autosomal Dominant
MR activating mutation	S810L	Autosomal Dominant
PHAII	WNK1 WNK4	Autosomal Dominant

Table I- Monogenic forms of hypertension
Examples of monogenic forms of hypertension
Descriptions of diseases and table based on Tanira et al (2004) and Lifton et al (2001)

The above mentioned forms of hypertension affect a common pathway via the control of salt regulation and salt re-absorption in the kidney, which indicates a key role of the kidney in the pathophysiology of hypertension. Extensive data from experimental animals suggest that hypertension cannot be sustained without active participation of the kidney (13). In addition, marked elevation of blood pressure in response to increased dietary salt intake has been observed in humans, primates and rodents (14), indicating the importance of inherited variation in renal salt handling. The relation between genetic variation and monogenic forms of hypertension provides insight into the more common forms of hypertension, especially in patients where the genetic defect has been characterized.

1.2.3 Essential Hypertension

Essential hypertension constitutes the vast majority of hypertension cases. It is a multifactorial form of the disease that implicates such determinants as genetics and environment. Risk of hypertension tends to concentrate itself in families and a strong aggregation of high blood pressure is observed in most of these families. Given the fact that the same family not only shares environment but their genes, it makes it very difficult to separate these two influences. Moreover, blood pressure is a complex trait and the clinical phenotype of high blood pressure can be observed in a variety of pathophysiological mechanisms.

It is now widely accepted that this type of hypertension is polygenic, this means that it is caused by a complex interaction between many genes. Essential hypertension would therefore be determined by the interaction of a few major genes or many minor genes. Depending on the combination of genes, different interactions would modulate the determination of blood pressure and the genetics would influence these interactions.

Most of the traits that have a significant impact on the health of a population implicate many genes which interact amongst themselves. Most of the time these genes are also influenced by environmental factors.(Figure 2) Very rarely do forms of this disease implicate only one gene. Very often, forms of this disease undergo a very rigorous regulation by a multitude of genes. Furthermore, this regulation could vary from one individual to the other, depending on the variety inherited. This renders the task of identifying the exact causes of essential hypertension much more difficult.

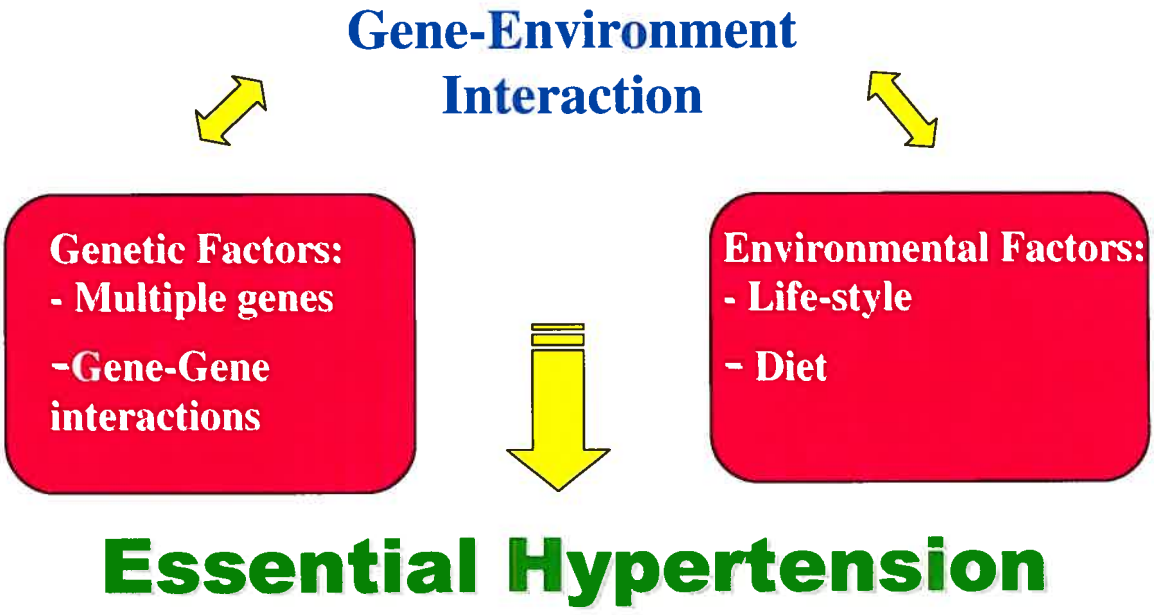


Figure 2- Gene-environment interaction
Figure representing the interactions of the many factors influencing hypertension.

1.3 Hypertension and Environment

Many genetic and environmental factors affect hypertension. Examples of such factors would be: sex, age, race, diet, consumption of alcohol or tobacco etc. The individual effect of genes can be additive or more complex and epistatic. As mentioned previously, the gene-gene interactions and gene-environment interactions, including the relation between these two groups render the study of this trait very complex.

It has been estimated that the genetic factor contributes from 30 to 50% of the variation of blood pressure which is observed. The identification of the genes that affect hypertension lead to a better comprehension of the pathophysiology of this disease. This information could then be useful to identify the individuals susceptible to this disease before the physiological manifestation, thus creating a preventive therapy. The knowledge of the genetic basis of this disease will also provide a better treatment by prescribing medication that responds to the specific needs of the affected individual.

1.3.1 Hypertension in industrialized populations

Many studies have shown a correlation between blood pressure and the location of inhabitance. Individuals that live in industrialized areas have blood pressure that increases which age. On the contrary, individuals living in non-industrialized areas, have a blood pressure which remains constant (15).

Stress is another environmental factor in industrialized populations which is important in the development of hypertension. It has been demonstrated that hypertensive or pre-hypertensive subjects, with a family history of positive hypertension, have a greater increase in blood pressure when they are exposed to a physical or mental stress than subjects which are of normotensive progeny with the same basal levels of blood pressure (16). A prolonged exposure to a chronic elevated stress throughout a long period of time could cause an anatomical adaptation in the heart and vessels and would therefore contribute to maintain a high blood pressure (16).

Many response genes could also be implicated in the environmental susceptibility to hypertension. Two such genes in the rat are *hsp70* and *tnfa*. Temperature and immobilization of the animal have been associated to changes in blood pressure and expression of these genes (17).

1.3.1 Hypertension and obesity

The association between hypertension and obesity has been well documented. It has been demonstrated that blood pressure is closely linked to body mass(17). This relation is valid for various populations and is confirmed not only in children but teenagers and adults as well. Studies have shown that nearly half of the population which is considered medically obese is hypertensive (18). Obesity brings upon physiological changes such as an augmentation in the resistance of the blood vessels and a cardiac hypertrophy (18). It has also been observed that a reduction of intra-abdominal fat is linked to a decrease in blood pressure in patients which are hypertensive and obese.

1.3.2 Consumption of alcohol and hypertension

There exists a correlation between alcohol and a high arterial pressure. Even today, there is no known signaling pathway which could clearly explain this link. In the Caucasian population, men have a diastolic and systolic pressure that increases with the amount of alcohol consumed. This tendency is also observed in women but the increase in arterial pressure is less pronounced (19). In the African-American populations, the men had a constant increase of diastolic as well as systolic pressure for the same alcohol consumption.

1.4 Salt sensitivity and hypertension

The amount of salt in one's diet is an important environmental factor in the regulation of blood pressure (20). It has been observed that the prevalence of hypertension is low in certain primitive societies with a diet which is feeble in salt (21). In the 1940's, it was demonstrated that a low-salt diet could reduce blood pressure in patients suffering from severe hypertension. Also, in certain patients (20 to 40%), a low-salt diet could help them to better control blood pressure (21).

Normotensive and hypertensive subjects can be either sensitive or resistant to the effects of an increase in blood pressure which is due to salt. Meaning that their blood can be sensitive or not to the effect of salt intake. In hypertensive subjects, those that were salt-sensitive had an elevation in blood pressure during a period of 24 hours, whereas the subjects which were salt-resistant had a high blood pressure only during their sleeping period. The kidneys have been long suspected of playing a central role in salt sensitivity and arterial pressure. It has also been demonstrated that salt sensitivity could also be modified by the presence of other ions in the diet.

1.4.1 Importance of ion transportation

At the base, essential hypertension is created by an imbalance between the peripheral resistance of the blood vessels and the blood volume. The mechanisms which are responsible are the regulatory contraction cells of the smooth vascular muscle in the arteries which forms the resistance and the regulation of extracellular fluids. It has been demonstrated that the response of the smooth muscle cells would play a role in the regulation of blood pressure (22).

The peripheral resistance and the regulation of the extracellular volume can be linked to the membrane which separates the extracellular and intracellular spaces. The structure and function of this membrane plays a key role in the pathogenesis of hypertension. The plasmic membrane is a dynamic structure that maintains its interactions with adjacent cells with the help of specific signals from the entire organism

1.4.2 Role of sodium

There exists a relation between the consumption of sodium and the development of hypertension in certain animal models and patients suffering from salt-sensitive hypertension. Cellular sodium is increased in many types of cells (erythrocytes, lymphocytes) in essential hypertension. This is due either to an increase or decrease in the entry of sodium. The principal determinant of this unequal distribution of sodium between the intracellular and extracellular compartments is the ATPase NA+K+ pump which exports three sodium ions for two potassium ions imported into the cell. This maintains a low concentration of intracellular sodium and a high concentration of potassium. This unequal distribution is responsible for the inflow of other ions between compartments. It is also possible that this pump is barred in certain forms of hypertension which are characterized by a large quantity of sodium and water.

1.4.3 Role of intracellular calcium

Intracellular calcium is a major determinant in the contraction of the smooth vascular muscle. It is also a key element in the cellular response to agonists, intracellular calcium acts a second messenger. In resting cells, it is kept constant by a variety of mechanisms which are mediated by sodium pumps located in the intracellular organelles. In humans and experimental models affected by hypertension, calcium homeostasis is affected.

The essential components of calcium homeostasis are the plasmic calcium ATPase, which pumps the calcium out of the cell and the sodium-calcium exchanger (NCX) which pushes the calcium out against its gradient in exchange with sodium which is pumped with its gradient. An equilibrium between these two mechanisms maintains an intracellular concentration of calcium which is four times weaker than the extracellular concentration. It has been demonstrated that entry of calcium via the type 1 NCX exchanger (NCX1) is implicated in the contractile regulation of the small arteries as well as in the development of salt-dependent hypertension. (21)

Chapter 2

Genetic approach

It's clear at the present time that essential hypertension is a polygenic and hereditary disease. Studying the genetic bases of hypertension is a very difficult task due to the fact that there are many genes involved each having a partial effect on blood pressure. The human population is genetically heterogeneous, each individual possesses a particular version of each of his or her genes and the combination of these genes renders each person unique. This phenomenon complicates the research of the responsible genes for this complex trait. Given the fact that each individual possesses slight variation differences for each gene, it is difficult to discover a small difference in the genome of a varied human population.

The main strategy used to study the genes implicated in hypertension is the identification of QTLs (quantitative trait loci) which are responsible for the variations of arterial pressure observed. The genes which contribute to a complex quantitative trait are known as QTL (23), the quantitative trait of hypertension being arterial pressure. The units used to measure arterial pressure are mmHg also known as millimeters of mercury. Due to the fact that a QTL is responsible for only a fraction of the trait observed, the phenotype-genotype correlation is weak (24).

An epistatic interaction happens when the combined effect of two or more genes cannot be predicted by a simple addition of their effects (25).An example of an additive effect would be on Chr. 10 in the Lewis rat where 3 QTLs were found to interact additively. (59) The influence of epistatic genes on complex traits is not very well understood due to the complexity of the studied traits. A more precise definition for an epistatic interaction was given by Bateson: an epistatic interaction happens when a gene interferes with the phenotype of another gene which is situated on another allele. In short, the phenotype is determined by the first gene and not the second.

There are only a small number of epistatic interaction cases in the study of complex traits such as QTLs. This is due to the analysis tools which are used. The majority of these tools calculate the general effect of the QTL on the phenotype and not the interaction effect of many QTLs (26).

Even with the difficulties encountered in epistatic interactions, there do exist some examples. Ohno et al. demonstrated the presence of epistatic interactions between hypertension and the SrcaII (sarco (endo) plasmic reticulum Ca2+-dependant ATPase II) (27). Rapp's team also demonstrated the possibility of an epistatic interaction between two arterial pressure QTLs in the rat (28). They located a QTL on both chromosome 2 and chromosome 10. Using genetic linkage studies, they observed a weak segregation of the F2 population between the QTLs of chromosome 2 and chromosome 10. To prove the presence of an interaction between these two QTLs, they constructed a double congenic. A double congenic is a congenic strain were two regions are targeted: the genomic background being from one strain, and the QTL region of chromosome 2 and 10 have the genotype of another strain. The QTL of chromosome 2 increased arterial pressure versus the parental hypertensive strain. Logically, one would think that the combination of these two QTLs in the same congenic strain would have an amplifying effect on the arterial pressure. In other words, the QTL of chromosome 2 increases the arterial pressure by 8 mmHg, the QTL of chromosome 10 increases arterial pressure by 15mmHg, if the interaction of these two QTLs is additive we expect to observe an increase of 23mmHg of the arterial pressure. Surprisingly, an augmentation of 47 mmHg was observed (29). This demonstrates that the –BP (blood pressure lowering) QTL is epistatic to the +BP (blood pressure raising) QTL.

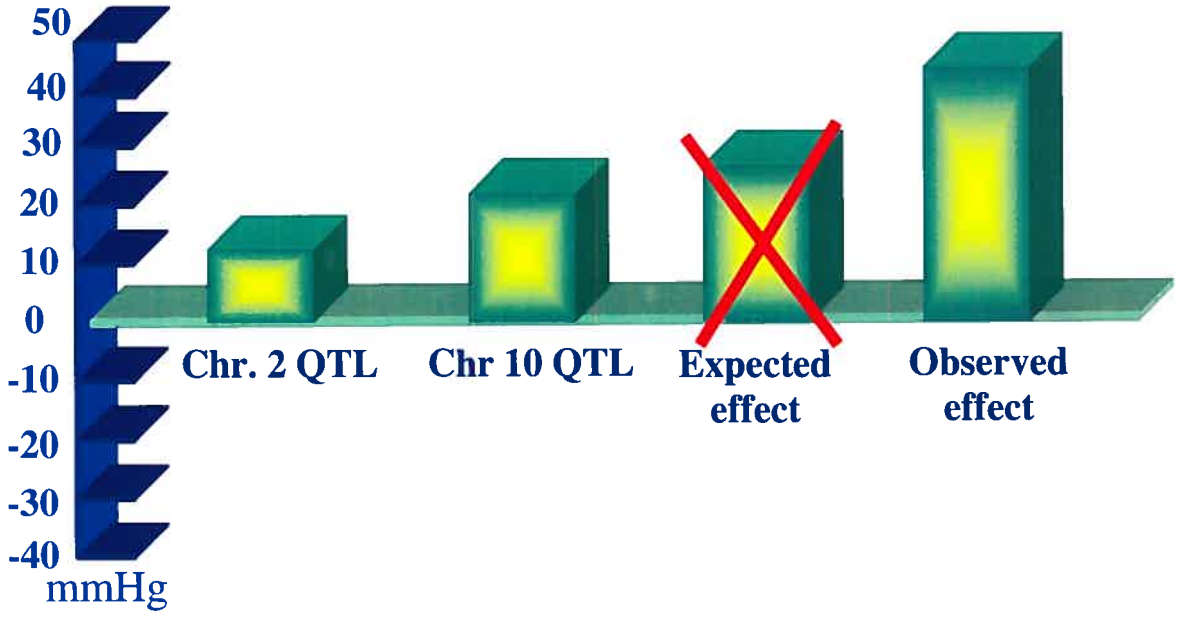


Figure 3- Epistatic effect of Chr.2 BP QTL and Chr 10 BP QTL
Representation of arterial pressures and observed effect as published in *Rapp et al.* (28)

Advancement in the domains of molecular biology and genomic techniques, in combination with those of physiology has given birth to functional genomics. Functional genomics is defined as a multidisciplinary approach in the study of genes, their products and interactions (30). Other strategies have also been put into place to identify the genes which are susceptible to hypertension (31).

Such strategies are:

1. Candidate gene approach
2. Linkage analysis
3. Comparative genomics
3. Animal models

2.1 Candidate gene approach

Most genetic studies implying hypertension-predisposing genetic loci have used this strategy to study candidate genes. This systematic approach assumes that a gene or a set of genes involving a specific physiological or cellular function contribute to blood pressure variation.

Employing this approach, some candidate genes have been elucidated from the study of rare monogenic forms of hypertension (Liddle's syndrome). For essential hypertension however, at least 51 genes/loci that affect different physiological or biochemical systems have been describe (32). Most of these studies utilizing genetic linkage and association methods enrolled unrelated individuals in case-control designs. Although case-control design is useful in homogeneous populations, these studies were performed in heterogeneous populations and thus may have higher probability of errors due to differences in the studied population (33). Case-control design also renders candidate gene studies difficult to be reproduced and less likely to include all causative genes and polymorphisms.

The candidate gene approach is limited by our knowledge of the pathophysiology of the disease in question; we are limited by the known genes that contribute an effect on the complex trait. This approach does not permit us to identify new genes implicated in the pathogenesis of the studied disease.

2.2 Linkage analysis studies

This method gives estimates for the genetic parameters behind any trait(s) being studied and proposes a model to explain the inheritance pattern of phenotypes and genotypes observed in a pedigree. It provides information on gene frequency, mode of inheritance and phenotypes.

It is also a common method used for genetic mapping. It uses a DNA polymorphic marker situated in proximity to a locus causing a disease, and this same locus will be followed during the segregation of the chromosomes in the affected individuals. This way a LOD score can be determined and linked to a specific gene which may cause disease. The two types of DNA polymorphisms that can be eveluated are RFLP (restriction fragment length polymorphisms) and VNTR (variable number of tandem repeats). VNTRs are constituted by a variable number of sequence repeated nucleotides in individuals. This sequence can be short (microsatellites and SSLPs-simple sequence length polymorphism) or long (minisatellites). In order to determine which polymorphism was inherited by each individual we use PCR (polymerase chain reaction) and gel electrophoresis to detect the repetitions and measure their different lengths. In the case of genetic linkage studies, microsatellite markers are the most frequently used.

The more a locus marker and a locus causing disease are in proximity to one another, the more they will travel together and have less recombinance between them. When these two loci follow one another more often than usual during segregation they are considered to be "paired" or linked. This linkage is quantified with the help of a *LOD score*. This is a measure of the probability of a linkage between the disease and the locus. Generally, a *LOD score* of 3 is necessary to be deemed significant and indicates a linkage probability of $p<0.05$ between these two loci. (34)

The results of genetic linkage analysis studies are presented in the form of a graphic where the curve represents the *LOD score* with each of the markers tested all along the length of the chromosome. When a significant peak is observed in the graph, the region which demonstrates this peak contains a QTL (quantitative trait locus). The localization of a QTL by linkage analysis determines a chromosomal interval of approximately 20 to 30cM (35). This constitutes a very big region and other methods must be used to confirm the QTL and further narrow the number of candidate genes (36). There is also a possibility that there are many QTLs located on the same chromosome. If the distance separating 2 QTLs is superior to 80cM, these two can then segregate independently which will be illustrated by two peaks in the graph (37). On the other hand if the distance separating the two is inferior to 80cM, two peaks will not be observed and the presence of two QTLs will be undetectable.

Genetic linkage analysis studies are mostly used when studying diseases implicating only one gene, such as monogenic forms of hypertension. In these studies a mendelian form of transmission is observed and one responsible gene is followed in affected families. Given the fact that the effects of this gene are present or absent in certain family members, the co-segregation of alleles causing the disease can be detected.

This type of analysis is very difficult to use for essential hypertension because it is the result of many genes. The contribution of each gene is weaker and detecting a link between alleles causing disease can be more difficult.

2.3 Association analysis

Association analysis is an alternative method to genetic linkage which compares the allelic frequency between the affected and non-affected individuals. If an allele is found more often in affected individuals than in non-affected individuals this allele is thought to possibly be linked to disease. These studies are more effective than linkage analysis in studying hypertension because they have greater statistical power to detect several small genes for a small effect (37), but they also have a greater tendency to yield false positives.

2.4 Comparative Genomics

This approach employs data from animal studies to identify potential blood pressure regulating loci in humans. In genetics, a good method to link the animal model to the human is by constructing a homology map. A homology map is constructed by placing homologous genes (found on NCBI) from different species on a map and comparing their function from one species to another. If the function and importance of the gene is conserved then that gene and its effects are further studied in depth.

This approach permits one to target potential QTL regions between species. More than 70 studies of human populations have identified QTL regions in the human genome. When animal models are used, a genetic comparison must be applied to be able to locate the homologous regions in the human genome. This method also permits us to confirm the results observed in the animals. In other words when a QTL region is localized in the animal genome, the conservation of the QTL can be studied by verifying if there is a superposition of the studied QTL with one which is already identified in the human genome (figure 4).

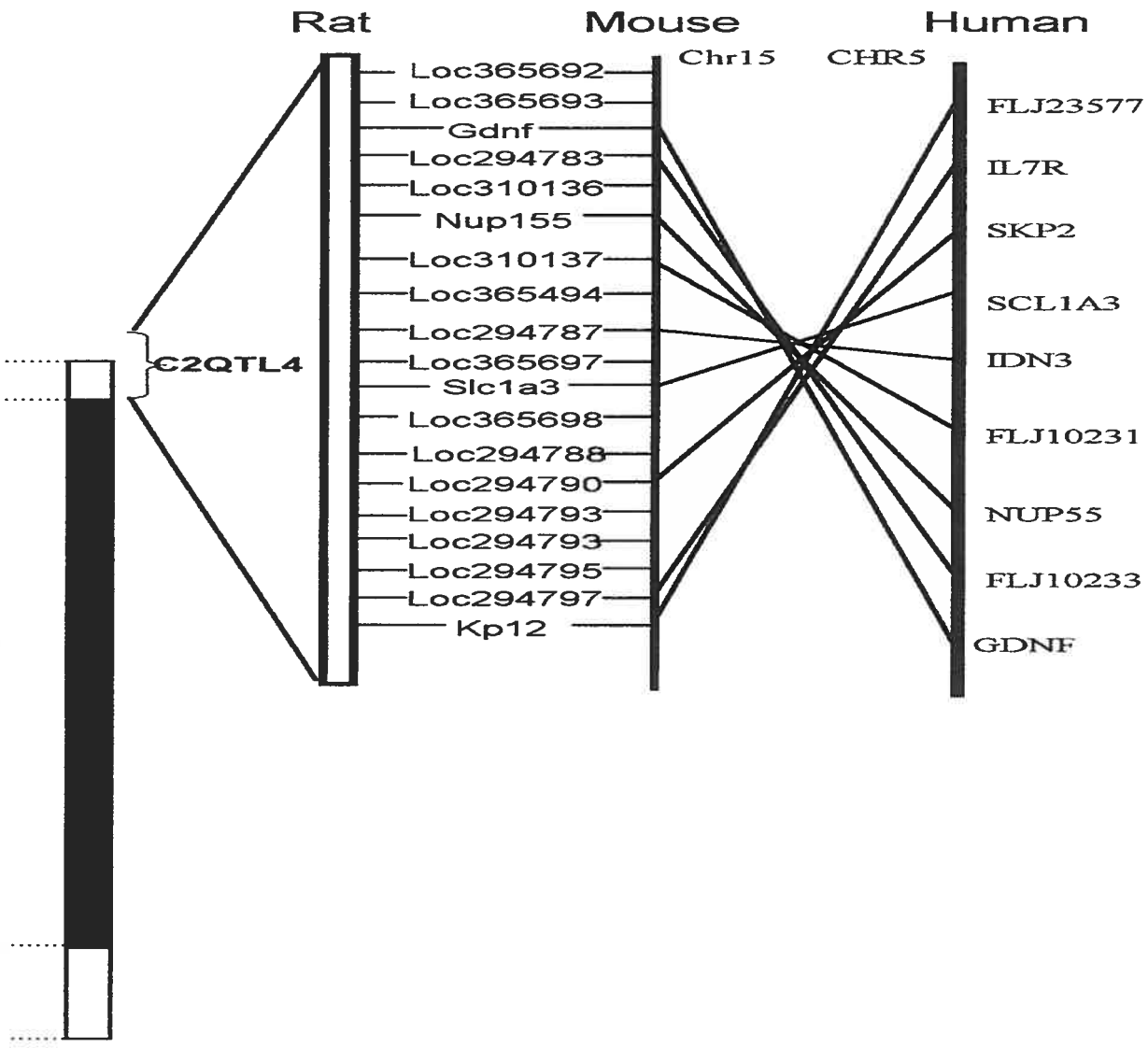


Figure 4- Homology map between the mouse, rat and human.
This figure demonstrates the conservation of the homologous regions and the localization of the QTL between species. This figure is taken from *Eliopoulos et al* (52).

2.5 Genomic Resources

Genomic resources are essential for the genetic analysis of a QTL. Many genomic resources have been put in place in the last few years with aim to facilitate these genetic analyses. The most important being the sequencing of the rat genome which was completed in 2003.

The sequencing of the rat genome was completed in 2003 with more than 90% of the genome sequenced. This sequence is now readily available at NCBI's website (http://www.ncbi.nlm.nih.gov/). In April of 2004, an article published in Nature explains the key elements to this sequencing. The Rat Sequencing project, also known as RGSP, started many years ago. Due to the fact that the rat is often used in many physiological and pharmacological studies, there was indeed a real need to know its genomic sequence even thought the rat and mouse are morphologically very similar and close ancestors from an evolutionary point of view.

Contrary to the genomic sequence of the mouse and human, which are entire sequences, the sequencing of the rat genome is not entirely completed. With sufficient time and funding it should be completed in the near future. This is significant and for this reason it is very important to keep a high standard in the quality of sequencing in order to minimize errors.

The rat genome measures 2.75Gb which is inferior to that of the human genome which measures 2,9Gb but larger than that of the mouse which measures 2.6Gb. It is important to remember that the assembling of the genome predicts a size that is always inferior to the actual size. This is mainly caused by the difficulties of sequencing as well as assembling the sequenced pieces (38).

2.6 Animal models

There exist animal models which were specially generated to represent human diseases such as hypertension. An animal model which suffers from hypertension is less complex to use. Contrary to human subjects, an animal strain can be crossed in such a manner that the differences in the genes solely related to hypertension can be studied. Also, animal models can be analyzed in a controlled environment where many variables, such as population and environmental conditions, can be pre-determined (39). Animal models also offer the possibility of a short gestation period as well as a large number of crosses and progeny which render the study of complex genetic phenotypes and gene-gene interactions easier to study.

2.6.1 The rat

Many rat and mouse models were established to study complex genetic traits. Such models include consomic, congenic and finally recombinant models (Figure 5). These strains have been crossed between brother and sister for many generations (about twenty) in order to obtain a good homogeneity (40). The goal in doing these crosses is to create strains which are rich in alleles which code for high arterial pressure. In this manner, some strains are selected for their high arterial pressure while others are selected for their low arterial pressure (41).

Transgenic models permit the study of candidate gene function by altering the level of expression of the gene. The targeting of candidate gene expression can be specific or not (targeting of simple gene). The targeting technique of a simple gene permits us to abolish the function of one gene or to duplicate the gene of interest. The first application of the transgenic model for hypertension was in the mouse. Examination of candidate genes known for their implications in a signaling pathway which is implicated in the control of arterial pressure yielded an analysis of almost ten genes; these genes were part of the renin angiotensin system.

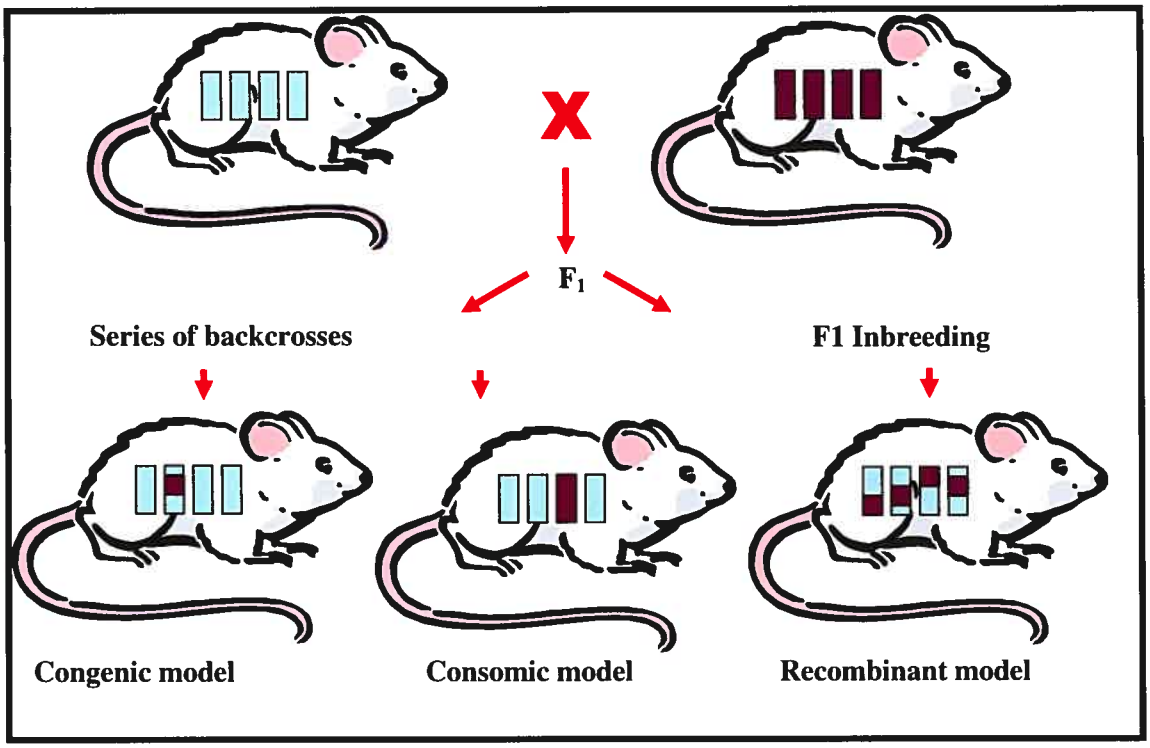


Figure 5 – Genetic tools using the rat
Based on a representation from McBride et al (2003) (48)

More and more researchers use animal models, such as the rat, to aid their research in hypertension. Today many rat strains exist and each offer their specific characteristics. Recombinant models are a useful tool in the localization of mendelian traits. Consomic and congenic models facilitate to target the chromosomal region which contains the QTL. In consomic models, a whole chromosome is substituted whereas in the congenic model only a segment of a chromosome is substituted. Consomic models are widely used to study the effect of the chromosome on arterial pressure. These strains, in the future, can then be converted to congenic strains. Congenic strains have the advantage of directly targeting the predetermined QTL region by using linkage analysis studies. The QTL regions can then be easily reduced by fabricating other congenic sub-strains.

Even though the rat has been known to transport dozens of diseases and is commonly known as a pest, it has contributed a great deal to humans and their health by helping us to better understand the complexity of human diseases. The laboratory rat also known as *Rattus norvegicus* originated from central Asia and is one of the first mammal species to be domesticated for scientific research in 1828 (42).The first genetic studies using the rat studied the color of their fur as a mendelian transmission trait (43). Even thought studies using rats had a very promising beginning, the mouse quickly became the model of choice for genetic studies in mammals. In contrast, the rat still remains the model of choice for research in physiology, nutrition, as well as other biomedical research where more than 235 rat strains are used.

The Dahl *Salt-Sensitive* rat (Table II) is an example of an available strain used in hypertension research. In 1963, Dahl started crossing rats which were selected for their sensitivity (S rat) and resistance to sodium (R rat) (47). Rapp then followed by creating strains which were a cross between brother and sister, therefore creating a prototype model for the study of hypertension (45). The S rats develop hypertension with a low salt diet but this hypertension is significantly augmented when a high salt diet is introduced (2 to 8% NaCl).

During the selection of rats which were sensitive to salt, other rats were selected specifically for their resistance to sodium. One such strain was the Lewis rat (Lew) which demonstrated a high resistance (46). This is surprising because Lew rats had previously not been selected for their resistance to sodium. In addition to having a high resistance to sodium, 45% of the microsatellites from the Lew genome are polymorphic with those of the S genome. In the case of the R rats, only 18% of the microsatellites were polymorphic with those of S (44). A larger percentage of polymorphic microsatellites increases the quality of genetic linkage.

Hypertensive or Normotensive Strain	Abbrev.	Origin	Reference
Genetically hypertensive	GH	Dunedin, New Zealand	Smirk et Hall, 1958[1]
Dahl salt-sensitive *Dahl salt-resistant*	S, DS, SS/Jr R, DR, R/Jr	Brookhaven, USA	Dahl et al., 1962
DOCA salt-sensitive (Sabra hypertensive) *DOCA salt-resistant (Sabra normotensive)*	SBH SBN	Jerusalem, Israel	Ben-Ishay et al., 1972[8]
Lyon hypertensive *Lyon normotensive* *Lyon low blood pressure*	LH LN LL	Lyon, France	Dupont et al., 1973
Spontaneous hypertensive rats	SHR	Kyoto, Japan	Okamoto et Aoki, 1963
Spontaneous hypertensive rats – stroke prone	SHRSP	Kyoto, Japan	Okamoto et al., 1974
Milan hypertensive *Milan normotensive*	MHS MNS	Milan, Italy	Bianchi et al., 1974
Fawn-hooded hypertensive *Fawn-hooded low blood pressure*	FHH FHL	Utrecht, Germany	Kuijpers et Gruys, 1984
Inherited stress induced arterial hypertensive	ISIAH	Novosibirsk, Russia	Markel, 1985
Prague hypertensive *Prague normotensive*	PHR PNR	Prague, Czech Republic	Heller et al., 1993

Table II- Strains of rats selectively developed for the study of hypertension
Table adapted from Rapp *et al* (47)

Methods

Chapter 3

Study of genetic linkage analysis

A genetic linkage study is based on the co-segregation of which occurs inside a certain population. To explain co-segregation and genetic linkage in mammal models, Rapp has given an example using 2 contrasting strains for each studied phenotype, which in this case is arterial pressure. In the example he uses, these are called P1 and P2 (47). The M marker is a microsatellite for the M locus which is linked to QTL A for arterial pressure (figure 6). The A1 allele of the P1 strain is the allele that diminishes the arterial pressure, whereas the A2 allele of the P2 strain is the allele that augments the arterial pressure. In this example, we have the M1 marker which is linked to QTLA on the A1 allele which diminishes arterial pressure and the M2 marker linked to QTLA on the A2 allele which raises the arterial pressure. Due to the fact that these two strains are produced from brother-sister crosses for many generations, the markers and loci are both homozygous for their alleles. Meaning that the studied gene or trait is followed through generations by following the specific markers which are linked to the alleles in that strain.

A large population is necessary to do a genetic linkage study. An F1 population is obtained by crossing the P1 and P2 strains. The F2 population is obtained by inbreeding the F1 population. The F2 population is then phenotyped for arterial pressure (in the case of our studies by telemetry) and genotyped using PCR with a microsatellite for the locus. During these crosses, the markers and alleles segregate using Mendel's theory (1:2:1). The arterial pressure observed is compared using statistic tools (such as ANOVA). In this example differences in arterial pressure will be observed because the marker is linked to a QTL for arterial pressure. The bigger the distance between the marker and the QTL, the higher the possibility of a recombinance. This diminishes the possibility of co-segregation of M with the arterial pressure.

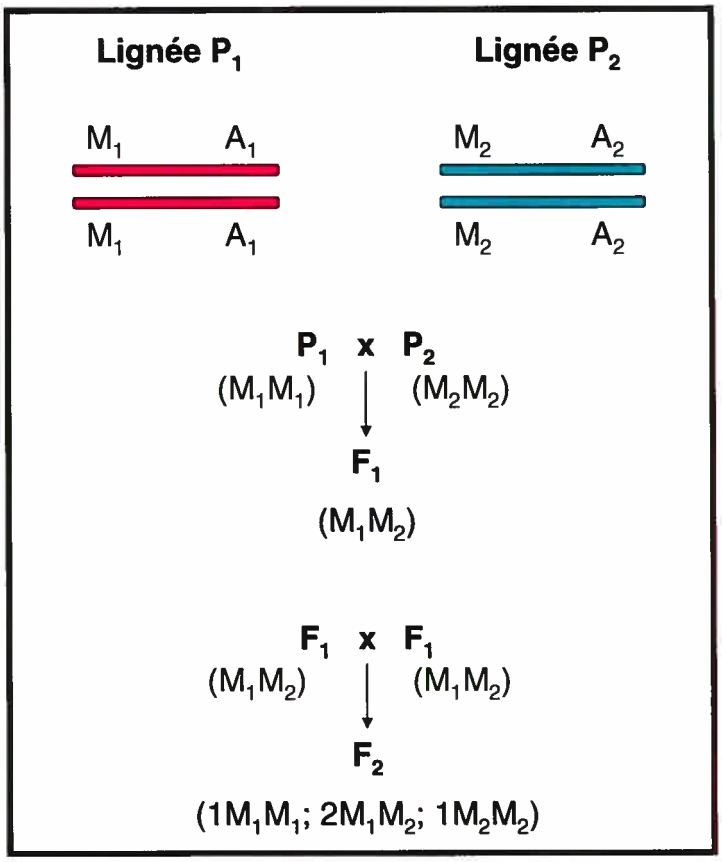


Figure 6- Co-segregation during linkage study
Picture based on a representation of article by Rapp et al.

The next step in a genetic linkage study is to localize the QTL on a chromosomal map and to identify the influence of this QTL on the arterial pressure. The potency of a QTL can bring upon difficulties in finding its exact position. For example, an effect on arterial pressure linked to a specific marker can be caused by a QTL which is situated in proximity to the marker used in the study, or to a QTL situated at a greater distance from the marker. This is due to the fact that the QTL's influence on arterial pressure is so large that it is observed to be linked to the marker which is situated farther.

Many statistical tools have been put in place to facilitate the localization of a chromosomal QTL. One such tool is MAPMAKER/QTL(48). This program predicts the possibility of the presence of a QTL by calculating logarithms where a result is obtained under a *LOD* connotation. A *LOD* is a log ratio of the possible presence of a QTL versus the possible absence of a QTL. The results obtained by these calculations are presentated in the form of a graph where the *LOD* is calculated at many intervals between the markers. The graph has the chromosomal positions on the *x* axis and the *LOD score* on the *y* axis. The interval where the *LOD score* is the highest is the chromosomal region which is the most susceptible of containing a QTL for arterial pressure. A *LOD score* higher than three is deemed significative. The interval of confidence is influenced by many factors such as the strength of the QTL on the observed phenotype, the size of the studied population and the density of the markers used.

Even if these factors are taken into consideration during the preparation of an experiment, the localization of a QTL by genetic linkage only limits us to a chromosomal interval of approximately 20 to 30 cM (49). Such a region contains hundreds of genes. Researchers then have to use to use another method to reduce the number of candidate genes.

Another problem which arises during genetic linkage studies is the analysis of information obtained from *LOD* curves. There is always a high possibility of the presence of many QTLs on a single chromosome. If the distance separating these two QTLs is superior to 80cM then there is a chance that these two QTLs segregate in an independent manner. This can be observed by two peaks on the graph. In the opposing case, if the difference separating the two QTLs is inferior to 80cM, the curve will not indicate the presence of many QTLs due to the fact that only one peak will be observed.

The presence of many QTLs on a single chromosome can also indicate a possibility of an interaction amongst them. If the interaction is additive, we may observe an amplification or a canceling out of the *LOD score*. In the first case, both QTLs have an effect of increasing arterial pressure then a high *LOD score* for a specific locus will be obtained. Therefore, by isolating the locus, there is a high possibility that's its effect on arterial pressure will not be as significant as previously noted. A canceling out of a *LOD* results from an additive interaction of 2 QTLs with opposite effects on AP.

Epistatic interactions are another form of interaction that QTLs may have. Such an interaction is common for complex traits but also difficult to identify due to the fact that it may give a non-significant *LOD score*. This kind of interaction will be explained in more detail further in this thesis.

Chapter 4

Congenic Strains

The presence of a blood pressure QTL on a chromosome cannot be proven simply by assessing a genomic study (50). With genetic linkage analysis studies, a very large QTL region is obtained. This region is too big to justify positional cloning and statistical analysis of these studies also present certain interpretational problems. Therefore, a physiological study must be done to show that the region predicted by the genomic study really does have an effect on arterial pressure. To accomplish this, we use congenic strains which permit the molecular study of these QTLs (51).

Congenic strains permit us to target a region of interest in the animal genome (Figure 6). The following example which was given by Rapp explains the construction of a congenic strain. The donating strain has a M1M1 genotype for the marker in the M locus. The receiving strain has a M2M2 genotype for the marker in the M locus. The receiving strain means that the genetic background of the congenic strains will come from this genotype and that this strain will accept the homologous segment of the donating strain. In this example, the goal is to obtain a genetic background of M2M2 genotype with a chromosomal M1M1 region. An F1 population is produced by crossing these two strains. This population will have a M1M2 genotype. The population is then crossed with the receiving strain. A population with the genotypes M1M2 and M2M2 will then be obtained. The heterozygotes will again be selected to be bred once more with the receiving strain. This backcross is done eight times in order to assure the homogeneity of the genetic background (52). Once the genetic background is homogenous, two M1M2 heterozygotes for the targeted region are intercrossed. We then obtain a population with a genetic background homogenous to the M2M2 genotype and having M1M1, M1M2 and M2M2 genotypes for the targeted region. The rats which contain M1M1 for the region of interest are then chosen for the quantitative studies of the studied trait.

Due to the many backcrosses, congenic strains take between two and four years to construct. With a larger amount of available markers in the rat, the amount of time necessary to construct congenic strains can be shortened by using the *speed congenics* method (53). This method uses markers dispersed throughout the genome, as well as the markers in the region of interest, to genotype the rats. This method permits to obtain congenic strains in a matter of 15 to 18 months (Figure 7).

Consequently, by constructing many congenic sub-strains for a QTL region we succeed in localizing the blood pressure QTL. Sadly, the smallest possible region we can identify using this method is 1 cM because the possibility of recombination between two nearby markers is low (54). Once the QTL region is reduced to 1cM, positional cloning can then start. Many articles have been published with QTL for blood pressure localized and reduced thanks to congenic strains (55-65).

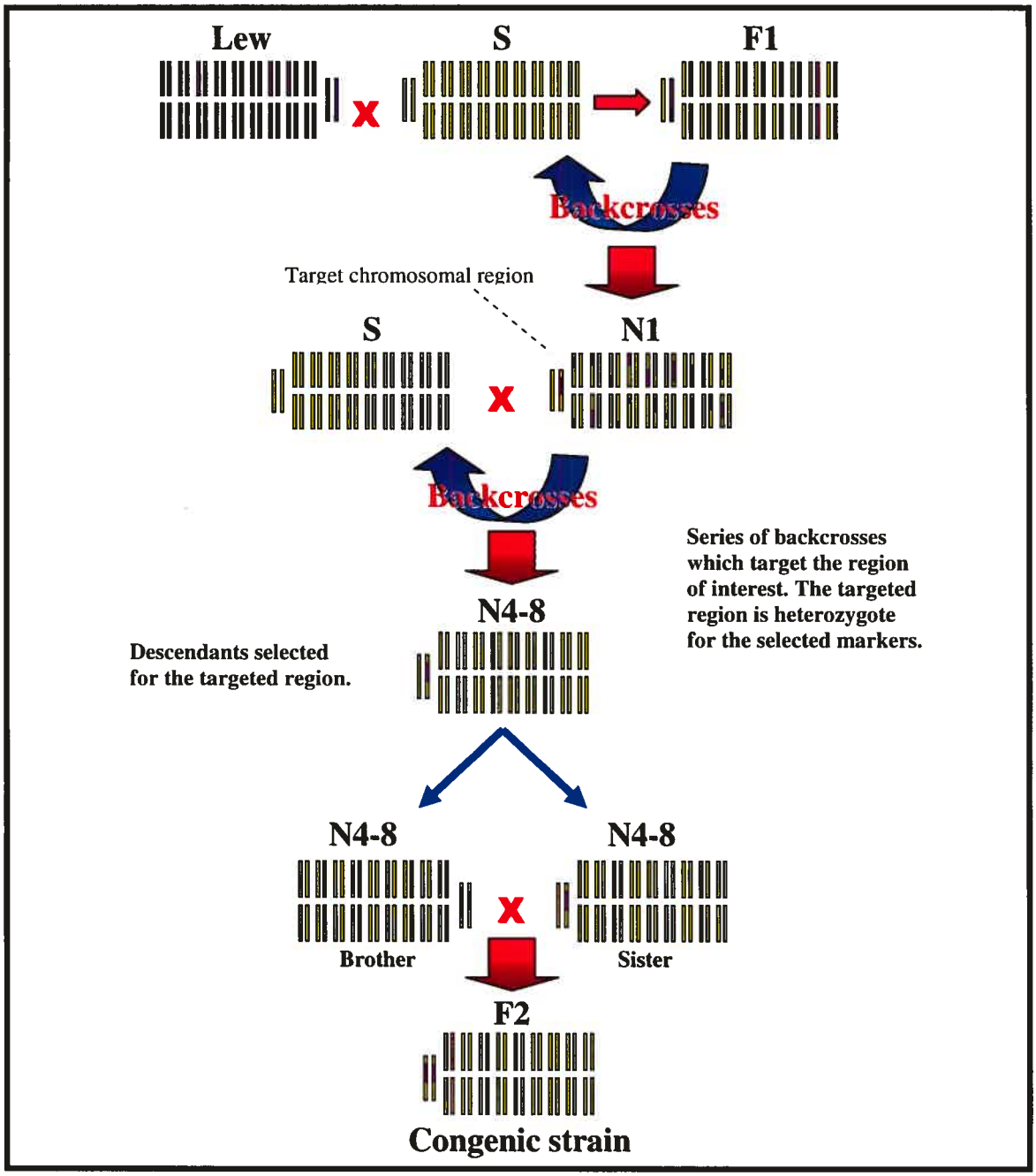


Figure 7- Schematic representation of the construction of congenic strains.
The parent strains S and Lew are crossed to obtain the heterozygous F1 population. This population is recrossed with th parental S strain. We then obtain the N1 population which is genotyped and the rats which are heterozygous for the markers flanking the chromosomal targeted region. A series of backcrosses are performed with these rats to obtain a population which is heterozygous (SL) for the targeted region and homozygous for the genetic background. The brothers and sisters having these characteristics will be crossed to obtain an SS descendance for the chromosomal background and LL for the targeted region. The rats are then crossed to obtain a stable congenic strain. Picture based on a representation from article by *Cowley Jr et al* (73)

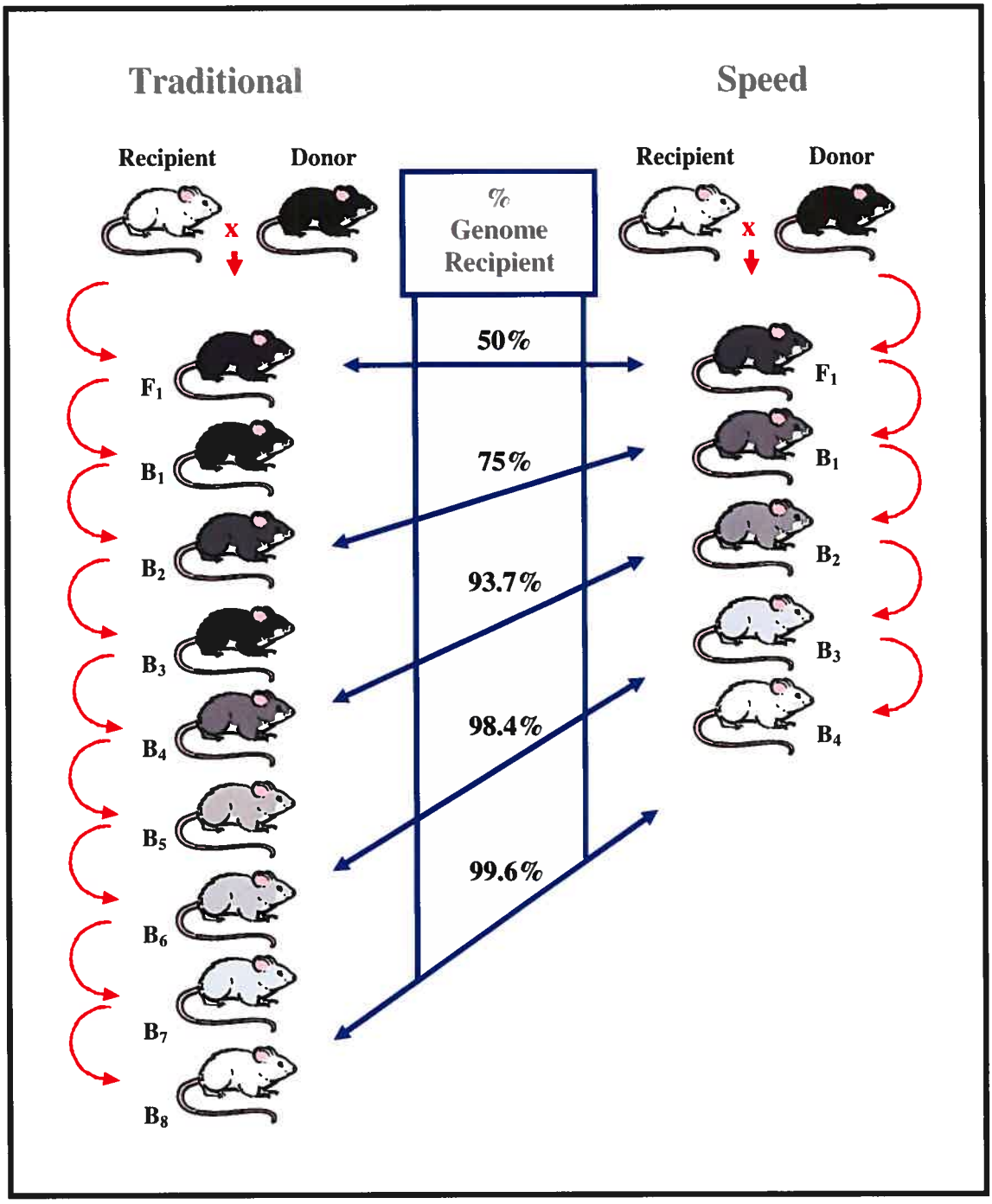


Figure 8- Method used to obtain rapid congenic strains (*speed congenics*)
The backross generations are reduced by approximately 50%. This approach uses polymorphic markers dispersed throughout the genome in equal intervals. Figure from *McBride et al.* (2003) (26).

Chapter 5

QTL Mapping

Before the sequencing of the rat genome, finding markers in the region of interest was more or less a question of chance. Many versions of genetic linkage and radiation hybrid maps have been published. These versions vary depending on the markers which are positioned and the distances between them. Therefore, the markers must not only be polymorphic but also situated at the right position and one has to integrate information from many maps.

This method is still widely used today but it is facilitated by the use of genomic sequencing. Thanks to this technique, it is now possible to position the marker on a chromosomal map with high precision. The main obstacle that one does face are the "holes" on a genetic linkage map (meaning a region on map where no polymorphic marker has been found). In the recent past the number of "holes" has been significantly reduced.

However, it is very important to note that the information obtained through the genomic sequence is not a certainty. By aligning the sequence of a marker with the available genomic sequence we obtain the markers position on the chromosome. Nevertheless, it is only by genotyping the marker using the congenic strains that we can confirm its position on the chromosomal map.

A genetic map which is rich in markers permits us to characterize the congenic strains with higher precision. This is accomplished by having a smaller distance between markers and therefore reducing the ambiguous regions. Smaller ambiguous regions in congenic strains facilitate the localization of the QTL by diminishing the QTL region. Many groups have demonstrated that a larger number of markers used in their studies have aided the localization of blood pressure QTLs. (66-68)

5.1 Microsatellites

Microsatellites are short tandem repetitions found throughout the non-coding genome of eukaryotes. These repetitions appear to be due to sliding during DNA replication (69). These repetitive sequences can be under mono-, di,-, tri or tetranucleotide form. Microsatellites vary in length and can be genotyped by a simple PCR reaction.

By comparing the human genome to the mouse genome, one notices that there are more microsatellites in the mouse genome. Also, these microsatellites have a tendency to be longer. On the other hand, when comparing the mouse and rat genomes, the differences are less noticeable.

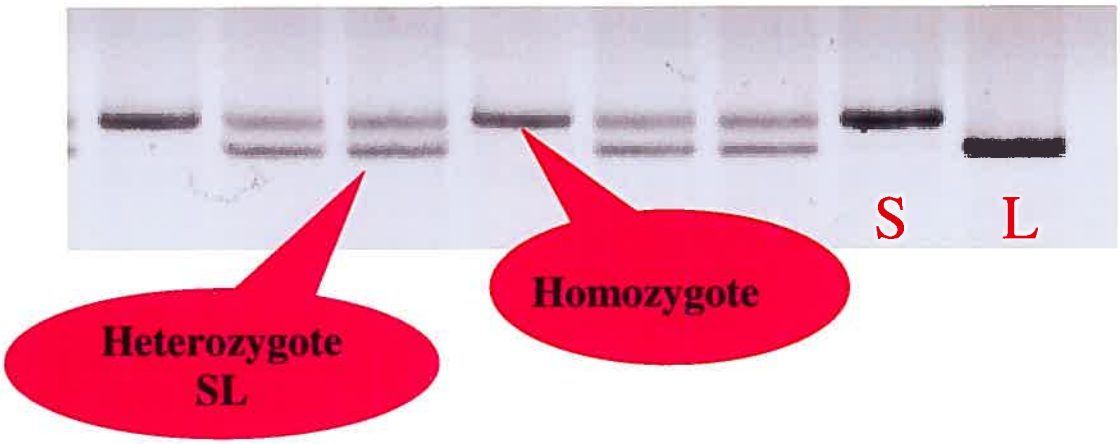


Figure 9-Example of PCR genotyping
An example of genotyping done by PCR demonstrating a heterozygote (SL) and a homozygote (SS) and controls on the right hand side.
Unpublished data V. Eliopoulos et al (2005)

With the sequence of most genes now available to any laboratory, the list of oligonucleotides used to amplify microsatellites by PCR have now become more extensive. Certain laboratories have now specialized in the identification and characterization of microsatellites and the synthesis of oligonucleotides which are necessary to amplify them. This work creates a large database for microsatellite use.

These microsatellites are in turn used to make genetic linkage maps. These maps have been established for each chromosome of the rat. These microsatellites allow researchers to place their target on a chromosome, thus calling them genetic markers. For a specific marker to be useful, it must be polymorphic for the strain of rat which is studied.

Many marker databases are available on the Internet (Table 3). This information can be under the form of a table or a map. In these tables or maps, one can find the approximate positions of the chromosomal markers. There are also databases which offer information on marker polymorphism, their optimal temperatures for PCR and finally the oliginucleotides used in PCR amplification.

Tools	Internet site
Genetic markers	http://ratmap.hgc.jp/Marker_search.html
	http://www.broad.mit.edu/rat/public/
	http://www.broad.mit.edu/rat/public/
	http://www.well.ox.ac.uk/rat_mapping_resources/markers_info/
	http://ratmap.org/ResultSearchLocus.htm?citno=666v
	http://www.well.ox.ac.uk/rat_mapping_resources/marker_polytest.html
	http://www.niams.nih.gov/rtbc/ratgbase/data/ARBPR3.htm
Genetic linkage maps	http://ratmap.hgc.jp/comp.html
	http://ratmap.hgc.jp/menu/map.html
	http://ratmap.org/ChromapnyPh.html
	http://ratmap.org/Idiogram.html
	http://ratmap.org/gene_mapping_data/integrated_linkage_maps/
	http://rgd.mcw.edu/tools/maps/maps_view.cgi?id=1006&chr=3
Hybrid radiation maps	http://www.well.ox.ac.uk/rat_mapping_resources/rat_RH_comprehensive_maps.html
	http://www.broad.mit.edu/rat/public/
	http://www.rgd.mcw.edu/tools/maps/maps_view.cgi?id=1002
	http://www.well.ox.ac.uk/rat_mapping_resources/rat_RH_framework_maps.html

Table III - Examples of Internet sites used to find marker information

5.2 Physical Maps

What was before considered a long and tedious task has now been simplified by the availability of the rat genomic sequence on the internet. Contrary to the genetic linkage maps or the hybrid radiation maps, where the distances are expressed in cM and cR respectively, a physical map gives the distances between markers and genes in pb.

Before the availability of the rat sequence, a physical map was nothing but an estimation of the distance between two markers. This approximate distance was obtained by aligning the bits of BAC and YAC which were available.

By having the larger fragments of the genomic sequence regrouped in supercontigs, determining the chromosomal position of a marker and the distance between two markers is much more precise. This can be accomplished by using a program called BLAST (http://www.ncbi.nlm.nih.gov/BLAST) which is available on the NCBI website.

Due to the fact that the genomic sequence of the rat was never really completed, some gaps or holes will always remain, thus leaving the possibility of errors. This can sometimes cause false results for certain alignments or even an absence of alignment for certain sequences.

5.3 Homology Map

A homology map has many functions, one of them being the identification of the chromosomal region of the human genome implicated in the trait studied and the second obtaining a more complete list of candidate genes.

To identify the region implicated in the human using rat studies, one establishes limits to each region. The alignment of sequences, as previously mentioned, is one of the methods used. It is important to remember that during evolution large chromosomal segments remained intact. This fact greatly facilitates the construction of a homology map. By aligning the sequences of the region of interest of the rat to that of the mouse genome, we then obtain a homologous region in the mouse. It is advised to first align the sequences of the rat and mouse and then those of the human because the first two are known to be evolutionary cousins.

Due to the fact that the genomic sequences are more complete in the mouse than the human, a homology map permits one to obtain a more precise list of candidate genes which are in the region of interest in the rat genome. The list of genes in the region of interest can be viewed by looking at the text version of the sequence on the NCBI site (http://www.ncbi.nlm.nih.gov/) or by using the MAPVIEWER program on this site. The MAPVIEWER program not only offers a visual version of the genome with the placement of genes, but also permits one to obtain information on the specific gene and its known homology in other species.

Results

Chapter 6

Previous studies

Michael R Garrett's team has previously done a genetic linkage study for essential hypertension by using two rat strains (70). In order for this study to be done, the two strains used must have contrasting phenotypes for the studied trait. Therefore, the Dahl *Salt-Sensitive* rat (S rat) and the Lewis rat (L rat) were used in their genomic study of the rat for blood pressure QTLs. In their study, a population of 151 rats were used as well as 406 markers which were spread out throughout the genome. These markers covered 97.3% of the genome and were separated by an average distance of 10cM.

For chromosome 2, 30 genetic markers were used to cover the length of the chromosome. Initial linkage studies indicated that a blood pressure (BP) QTL was probably present in a segment between D2Mit6 and D2Mco19 markers based on an F_2(DSS x LEW) population (71). The detection of this QTL was supported by a maximum *LOD score* of 2.9 (71), and therefore, was still below what was considered the highly significant level for detecting a QTL (*LOD score* of three) in such a population (72). An obvious question to ask oneself is 'could this detection be a true localization of a BP QTL?' Moreover, Stoll and coworkers (73) detected QTLs for several physiological phenotypes in the vicinity of the BP QTL on Chr 2 found in our F_2(DSS x LEW) population (71) . This region, therefore, could potentially harbor important genes not only for BP, but also for other physiological traits. Another question which remained was if the gene encoding the angiotensin receptor type AT1B (*Agtr1b*), which is included in this area, could represent a candidate gene for the QTL?

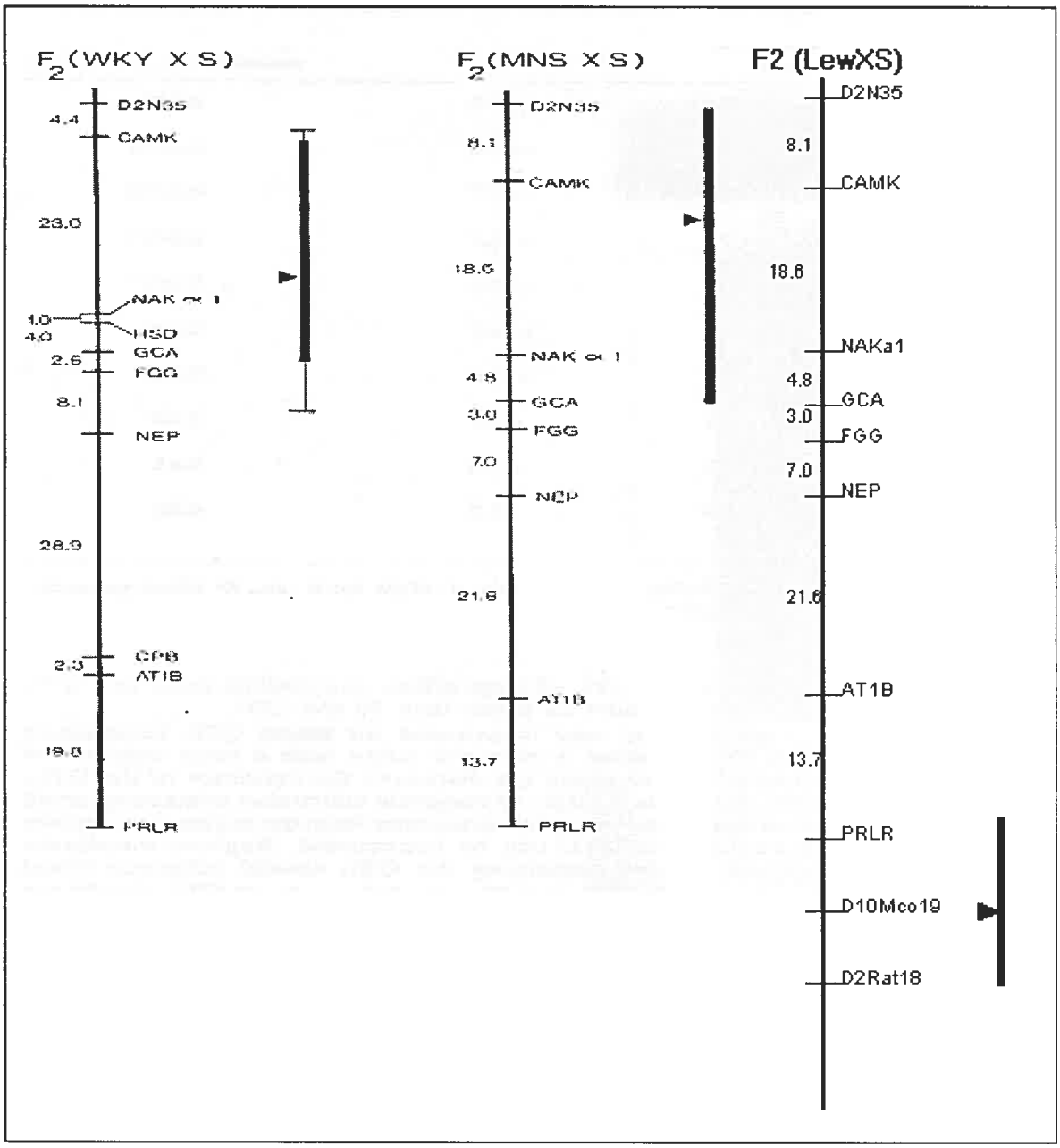


Figure 10-Previous linkage analysis studies

This figure demonstrates different linkage analysis studies which were done using various strains which localize the QTL in different regions depending on the strains studied. The F2 populations using the LEW and S rat were the ones which were of interest to us. This linkage analysis predicts a blood pressure QTL to be between the PRLR marker and D2Rat18. Taken from *Rapp et al* (70).

Chapter 7

Project

In order to find out if chromosome 2 does indeed contain a blood pressure QTL we used the congenic strain method. We constructed congenic strains using the same two strains used in the linkage analysis because variation can sometimes occur between the strains of rats used.

Four congenic strains, C2S.L1, C2S.L2, C2S.L3 and C2S.L4 (Figure1A), were constructed and purposely designed to overlap each other in chromosomal coverage, but in the meantime, leave no gaps uncovered for the entire region of interest predicted by previous studies. C2S.L3 was to include the gene *Agtr1b* for the specific purpose of testing its candidacy for a BP QTL. The arterial pressure of the rats was then measured using telemetry.

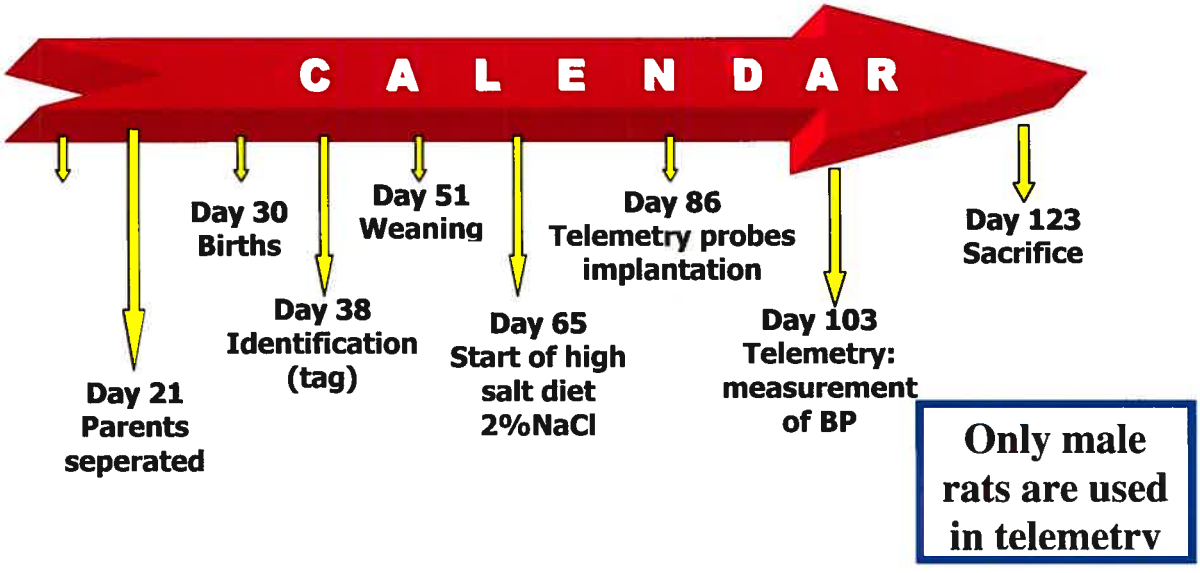


Figure 11-Telemetry
Example of timeframe and techniques followed in order to have consistent results in telemetry.

After constructing the first congenic strain (C2SL1) we had to further narrow the region, therefore we constructed C2SL2 and C2SL3. Once it was noted that the blood pressure of C2SL2 and C2SL3 did not vary from that of DSS, we then continued to construct C2SL4. In our studies we found that SAPs, DAPs and MAPs of C2S.L1 and C2S.L4 were higher ($p<0.003$) than those of DSS (Figure 2A). In comparison, MAPs, DAPs and SAPs of C2S.L2 and C2S.L3 were not different ($p>0.12$) from those of the DSS strain (Figure 2A). Consequently, the region containing the QTL, C2QTL4, can be localized to the segment that was in common between C2S.L1 and C2S.L4, but not included in C2S.L2 and C2S.L3 (Figure 1A). This segment is between D2Chm277 and *Prlr* (Figure 1A).The interval of D2Chm277/*Prlr* was calculated to be about 3 megabases (Mb).

What differentiates this study from others is the fact that in this QTL, the alleles of the QTL from the LEW rat actually raise blood pressure. This is contrary to common assumptions where the alleles of the LEW rat are normotensive. This is explained in better detail further on.

Severe hypertension caused by alleles from normotensive Lewis for a quantitative trait locus on Chromosome 2

Vasiliki Eliopoulos[1], Julie Dutil[1], Yishu Deng[2], Myrian Grondin[1], Alan Y. Deng[1]

[1]Research Centre- Centre Hospitalier de l'Université de Montréal (CHUM), Hôtel Dieu, 3840 rue, St. Urbain, Montréal, Québec, H2W 1T8, Canada

[2]Number three People's Hospital of Yunann, Kunming, Yunnan, China

Running head: QTL alleles from Dahl rats lowering BP on Chromosome 2

Corresponding author: Alan Deng, Ph.D., F.A.H.A.

Research Centre-Centre Hospitalier de l'Université de Montréal (CHUM)

7-132 Pavillon Jeanne Mance, 3840, rue St. Urbain

Montréal, Québec, H2W 1T8, Canada

Phone: 514-890-8000 ex. 15522

Fax: 514-412-7152

Email: alan.deng@umontreal.ca

Abstract

Pursuing fully a suggestion from linkage analysis that there might be a quantitative trait locus (QTL) for blood pressure (BP) in a Chromosome (Chr) 2 region of the Dahl salt-sensitive rat (DSS), four congenic strains were made by replacing various fragments of DSS Chr 2 with those of Lewis (LEW). Consequently, a BP QTL was localized to a segment of around 3 centiMorgan (cM) or near 3 megabases (Mbs) on Chr 2 by comparative congenics. The BP-augmenting alleles of this QTL originated from the LEW rat, a hypotensive strain compared to DSS. The dissection of a QTL with such a paradoxical effect illustrated the power of congenics in unearthing a gene hidden in the context of the whole animal system presumably by interactions with other genes. The locus for the angiotensin II receptor AT1B (*Agtr1b*) is not supported as a candidate gene for the QTL because a congenic strain harboring it did not have an effect on BP. There are approximately 19 known and unknown genes present in the QTL-interval. Among them, no standout candidate genes are reputed to affect BP. Thus the QTL will likely represent a novel gene for BP regulations.

Key words: comparative congenics, functional genomics, blood pressure, fine mapping

Introduction

Our initial linkage studies indicated that a BP QTL was probably present in a segment between D2Mit6 and D2Mco19 markers based on an F_2(DSS x LEW) population (16). The detection of this QTL was supported by a maximum LOD score of 2.9 (16), and therefore, was still below what was considered the highly significant level for detecting a QTL in such a population (19). An obvious question to pose is 'could this detection be a true localization of a BP QTL?' This issue is important because linkage analysis is usually the first step in localizing a QTL for complex traits on Chr2 (3; 6; 9-11; 24; 26; 27; 30). Moreover, Stoll and coworkers (29) detected QTLs for several physiological phenotypes in the vicinity of the BP QTL on Chr 2 found in our F_2(DSS x LEW) population (16) . This region, therefore, could potentially harbor important genes not only for BP, but also for other physiological traits.

Based on above considerations, there were several questions to be addressed: (a), Did a BP QTL exist in a region detected by linkage analysis (16)? (b), If it did, does LEW possess BP-raising alleles, and can the gene encoding the angiotensin receptor type AT1B (*Agtr1b*) be supported as a candidate gene for the QTL? The present investigation was intended to address these two questions and to fine map the QTL in question.

Materials and Methods

Animals and generation of congenic strains: The breeding procedure, markers used in genomic scans, and the screening protocol for generating congenic strains were essentially the same as reported previously for mapping BP QTLs on other chromosomes (2; 7; 20-23; 28). For our current work, four congenic strains were produced, and are designated as DSS.LEW-(D2Rat199-D2Rat143)/Lt (abbreviated as C2S.L1), DSS.LEW-(D2Rat18-D2Chm277)/Lt (C2S.L2), DSS.LEW-(*Prlr*-D2Rat143)/Lt (C2S.L3) and DSS.LEW-(D2Rat199-D2Mco17)/Lt (C2S.L4) respectively. C2 indicates that the strain is made for Chr 2. The chromosome regions homozygous LL in the congenic strains are shown as solid bars in Figure 1. All the markers in the region concerned were genotyped in the congenic strains.

Production of new markers: The process is similar to what was reported previously for other chromosomes (2; 21; 22). The PCR primers for the new D2Chm markers used in the present studies are given in the legend for Figure 1.

Breeding protocol for BP studies: The determination of BPs is essentially the same as described previously (2; 7; 12; 13; 20-23; 28). In brief, the mating pairs of the DSS and congenic strains to be studied were bred simultaneously. Male rats were selected and weaned at 21 days of age, maintained on a low salt diet (0.2% NaCl, Harlan Teklad 7034) and then fed a high salt diet (2% NaCl, Harlan Teklad 94217) starting from 35 days of age until the end of the experiment. Telemetry probes were implanted when rats were 56 days old (i.e. after 3 weeks of the high salt diet) with their body weights

between 250-320 grams. After the surgery, the rats were allowed 10 days to recuperate before their BPs were read. The implantation of telemetry probes, the age and postoperative cares of animal are the same as described before (12; 13).

BP measurements: The basic protocol was similar to our previous congenic work regarding the age and sex of the rats, and in terms of the timetable of dietary treatments (2; 7; 12; 13; 20-23; 28). One BP reading was taken every 2 minutes for the period of measurement. Then, these readings were averaged for 6 hours to obtain one data point, which appears as a point on the graph for the purpose of showing diurnal variations (Figure 2). Please see Dutil and Deng (12) for detailed comparisons in BPs of DSS and a congenic strain exhibiting even finer variations during our typical BP measurements.

Statistical analysis: Repeated measures' Analysis of variance (ANOVA) followed by the Dunnett test in the SYSTAT 9 program (SPSS Sci. Chicago, IL) was used to compare the significance level for a difference or a lack of it between a congenic strain and the DSS strain. The Dunnett test takes into account multiple group comparisons as well as sample sizes among the comparing groups. In the analysis, a BP component was compared at each day for the period of measurement among the strains (2; 7; 12; 13; 20-23; 28).

As BPs of rats were measured continuously for about 2-3 weeks and varied with time, the numbers given at the bottom of Figure 1 represented only averaged values of mean arterial pressures (MAP) for a strain, and did not reflect the day to day BP variations. A Dunnett value including '<' given in each comparison between a congenic and DSS strains was the most conservative p value among all the days of comparisons.

Results

Congenic constructions: A total of eight rats with various crossovers in the regions of interest were obtained. Among them, two rats died before yielding any strains and six rats eventually gave rise to six congenic strains. Among them, two contained chromosome fragments inside those of C2S.L2 and C2S.L3 (Figure 1). Since both C2S.L2 and C2S.L3 did not exhibit BP effects (Figure 2), these two congenic strains were discarded.

The four congenic strains, C2S.L1, C2S.L2, C2S.L3 and C2S.L4 (Figure 1), were designed to overlap each other in chromosome coverage, but in the meantime, leave no gaps uncovered for the entire region of interest. C2S.L3 was to include the gene *Agtr1b* for the specific purpose of testing its candidacy for a BP QTL.

BP Studies: All the BP components were measured including systolic (SAP), diastolic (DAP) and mean arterial pressures (MAP). BPs for all the strains shown in Figure 1 were measured at least at two different times, i.e. they were separate litters raised at various times during a period of one year. This consideration was designed to minimize the environmental influences on the phenotyping accuracy. The results showed that BPs for each strain, C2S.L1, C2S.L2, C2S.L3, C2S.L4 and DSS, were not different at the separate periods of measurements (data not shown). Therefore, the BP data for each strain were pooled from these reproducible measurements (Figure 2).

Mapping of a BP QTL by comparative congenics: SAP, DAP and MAP of LEW were lower ($p<0.001$) than those of DSS (Figure 2); in contrast, SAPs, DAPs and MAPs of C2S.L1 and C2S.L4 were higher ($p<0.003$) than those of DSS (Figure 2). In

comparison, MAPs, DAPs and SAPs of C2S.L2 and C2S.L3 were not different ($p>0.12$) from those of the DSS strain (Figure 2). Consequently, the region containing the QTL, C2QTL4, can be localized to the segment that was in common between C2S.L1 and C2S.L4, but not included in C2S.L2 and C2S.L3 (Figure 1). This segment is between D2Chm277 and *Prlr* (Figure 1).

The interval of D2Chm277/*Prlr* was calculated to be about 3 megabases (Mb) as follows (Figure 1). D2Chm277 is at the position of 1.9 Mb in supercontig 47621.1 (with 1.9 Mbs total), *Prlr* is at the position of 2.9 MB in supercontig 47622.1. The QTL interval would be 2.9 Mb plus a gap with unknown number of bases between the two supercontigs. Calculating from the distance of cM 12.7 divided by 13Mbs in physical distance between C2Mco13 and *Prlr* (Figure 1), 1 cM = 1 Mb. Thus, the C2QTL4 interval of 3 Mbs is about 3 cM.

Systematic comparative mapping in search of candidate genes: Possible genes in the QTL interval are included in Figure 1 by comparative mapping among the rat, mouse and human genomes at http://www.ncbi.nlm.nih.gov/mapview/. Figure 3 synthesizes the QTL localizations so far documented in the literature, and places the present QTL with reference to the others.

Discussion

Major findings of the current work are (a) LEW carries high-BP alleles and DSS carries low-BP alleles at C2QTL4, despite that LEW is a normotensive strain, whereas DSS is a hypertensive strain. The congenic strain, C2S.L1 and C2S.L4, can be classified as a 'DSS' strain with severe hypertension. (b) The position of C2QTL4 has been restricted to an interval of 3 Mbs (or around 3 cMs) by comparative congenics, i.e. comparing the two congenic strains that had, with two that did not have, BP effects. The QTL interval harbors 19 possible genes, 4 known and 15 undefined Locs (Figure 1). This size is amenable for positional cloning. (c) *Agtr1b* has been disqualified as C2QTL4 because C2S.L3 had a BP not distinguishable from that of DSS. Since no apparent candidate genes are found in the segment harboring the QTL, the QTL discovery will inevitably lead to the identification of a brand-new gene previously unknown to influence BP.

BP of DSS could be raised by substituting alleles of C2QTL4 by those of LEW (Figures 1 and 2). Although LEW contains C2QTL4 alleles that exert BP-elevating effects, paradoxically, BP of the LEW strain itself is considerably lower than that of DSS (Figure 2). This fact indicates that it is not a single QTL, but rather it is how this QTL interacts with others, that determine the overall BP of a strain. Among the mechanisms of interactions, one can be epistasis. For example, the two QTLs on Chr 3 possessed opposing BP effects, one decreasing and other increasing BP (22). Yet the combined effect of these two QTL was equal to that of the QTL that decreased BP (22). This epistasis implies that these two QTLs acted in the same pathway/cascade leading to BP determination (4; 5). In addition, the numerical advantage of BP-decreasing QTLs might

have outweighed and nullified the effect of fewer BP-increasing QTLs in LEW, since there have been more BP-decreasing QTLs found in LEW than in DSS (4).

C2QTL4 is localized to the Chr 2 segment between D2Chm277 and *Prlr* markers because C2S.L1 and C2S.L4 did, whereas C2S.L2 and C2S.L3 did not, have BP effects (Figures 1 and 2). This approach of comparative congenics in placing a BP QTL is consistent with our other work in mapping C10QTL2 and C10QTL3 on Chr 10 (23) and in mapping two QTLs in the lower part of Chr 2 (Figure 3). For example, using the same approach, we initially localized C2QTL1 to a 5.7 cM segment of none overlapping between a congenic strain that had and a congenic strain that did not have a BP effect on Chr 2 (13). Later, a congenic substrain specifically involving the 5.7 cM region proved that, indeed, there was a BP QTL in the region (14). In line with this expectation, an eventual proof that C2QTL4 is present in the fragment shown in Figure 1 will come from making a congenic strain that specifically involves the interval of D2Chm145/*Prlr*. A congenic substrain trapping the QTL with a 'minimum' chromosome coverage (e.g. 1 cM or less) is desirable not only for the proof of its existence, but also for its final molecular identification.

The possibility of finding more than one QTLs in the fragment between D2Chm277 and *Prlr* (Figure 1) can not be excluded. Only further fine congenic mapping can resolve this issue. There is evidence that more than one BP QTLs were present close to each other in the lower part of Chr 2 (Figure 3), on Chr 10 (23), on Chr 3 (22), on Chr 8 (2), on Chr 5 (17) and on Chr 1 (15; 25).

It is worth noting that *Agtr1b* is not supported as a candidate gene for the QTL because congenic strain C2S.L3 harbors it, and yet, did not show a BP effect (Figures 1 and 2). A thorough comparative mapping among the rat, the mouse and the human genomes did not reveal obvious candidate genes known to influence BP in the interval of D2Chm277 and *Prlr* containing the QTL (Figure 1). Thus, it is most likely that this QTL will represent a novel gene for BP regulation.

Since our initial work (6; 10; 16), rat Chr 2 has been shown to contain QTLs with BP-raising alleles originating from various hypertensive rat models (1; 3; 18; 24; 27; 30). These QTLs are mostly clustered in the region between D2Rat303 and D2Mgh12 markers in the lower part of the chromosome (Figure 3). C2QTL4 identified in our current work seems to be unique in its chromosome location and, in contrast to other BP QTLs, in its BP-decreasing effect from DSS.

The existence of C2QTL4 found by linkage (16) followed by the current congenic confirmation was in contrast to the statistical detection (6; 9) and subsequent non-confirmation (13) of another QTL near *Agtr1b* (9), which compared DSS to the Milan normotensive strain (MNS). The latter outcome could probably be attributed to a false positive of statistics in our linkage results in the F_2(DSS x MNS) population (6; 9). Thus, it is essential to verify the existence of a QTL by congenic strains after linkage.

In conclusion, there is a QTL(s) for which BP-raising alleles originate, paradoxically, from the normotensive LEW strain in stead of the hypertensive DSS strain on Chr 2. This QTL can act independently from other QTLs in the context of the DSS background. Genes located in the QTL interval are not known to affect BP. Therefore, the gene discovery on this QTL will unravel novel mechanisms controlling pathogenesis of hypertension.

Acknowledgements:

This work was supported by a grant from the Canadian Institutes for Health Research (CIHR) to AY. Deng. AY. Deng is an Established Investigator of the American Heart Association, National Center. J. Dutil holds a CIHR graduate fellowship. We thank Mr. Eric Martel for his computer program in finding microsatellites in the rat genome.

Reference List

1. **Alemayehu A, Breen L, Krenova D and Printz MP. Reciprocal rat chromosome 2 congenic strains reveal contrasting blood pressure and heart rate QTL. *Physiol Genomics* 10: 199-210, 2002.**

2. **Ariyarajah A, Palijan A, Dutil J, Prithiviraj K, Deng Y and Deng AY. Dissecting quantitative trait loci into opposite blood pressure effects on Dahl rat Chromosome 8 by congenic strains. *J Hypertens* 22: 1495-1502, 2004.**

3. **Bilusic M, Bataillard A, Tschannen MR, Gao L, Barreto NE, Vincent M, Wang T, Jacob HJ, Sassard J and Kwitek AE. Mapping the genetic determinants of hypertension, metabolic diseases, and related phenotypes in the lyon hypertensive rat. *Hypertension* 44: 695-701, 2004.**

4. **Deng AY. Functional genomics of blood pressure determination: dissecting and assembling a polygenic strait by experimental genetics. *Current Hypertension Reviews* 1: 35-50, 2005.**

5. **Deng AY. In search of hypertension genes in Dahl salt-sensitive rats. *J Hypertens* 16: 1707-1717, 1998.**

6. **Deng AY, Dene H and Rapp JP. Mapping of a quantitative trait locus for blood pressure on rat chromosome 2. *J Clin Invest* 94: 431-436, 1994.**

7. **Deng AY, Dutil J and Sivo Z. Utilization of marker-assisted congenics to map two blood pressure quantitative trait loci in Dahl rats. *Mamm Genome* 12: 612-616, 2001.**

8. **Deng AY, Jackson CM, Hoebee B and Rapp JP. Mapping of rat chromosome 2 markers generated from chromosome-sorted DNA. *Mamm Genome* 8: 731-735, 1997.**

9. **Deng AY and Rapp JP. Evaluation of the angiotensin II receptor AT1B gene as a candidate gene for blood pressure. *J Hypertens* 12: 1001-1006, 1994.**

10. **Deng Y and Rapp JP. Cosegregation of blood pressure with angiotensin converting enzyme and atrial natriuretic peptide receptor genes using Dahl salt-sensitive rats. *Nature Genetics* 1: 267-272, 1992.**

11. **Dubay C, Vincent M, Samani NJ, Hilbert P, Kaiser MA, Beressi JP, Kotelevtsev Y, Beckmann JS, Soubrier F, Sassard J and Lathrop GM. Genetic determinants of diastolic and pulse pressure map to different loci in Lyon hypertensive rats. *Nat Genet* 3: 354-357, 1993.**

12. **Dutil J and Deng AY. Further chromosomal mapping of a blood pressure QTL in Dahl rats on chromosome 2 using congenic strains. *Physiological Genomics* 6: 3-9, 2001.**

13. **Dutil J and Deng AY. Mapping a blood pressure quantitative trait locus to a 5.7-cM region in Dahl salt-sensitive rats.** ***Mammalian Genome*** **12: 362-365, 2001.**

14. **Dutil J, Tremblay J and Deng AY. Constructions of non-overlapping congenic strains to prove the presence of two blood pressure QTL on rat Chromosome 2.** ***J Hypertens*** **20: S4, 2002.**

15. **Frantz S, Clemitson JR, Bihoreau MT, Gauguier D and Samani NJ. Genetic dissection of region around the Sa gene on rat chromosome 1: evidence for multiple loci affecting blood pressure.** ***Hypertension*** **38: 216-221, 2001.**

16. **Garret MR, Dene H, Walder R, Zhang Q, Cicila GT, Assadnia S, Deng AY and Rapp JP. Genomic scan and congenic strains for blood pressure quantitative trait loci using Dahl salt-sensitive rats.** ***Genome Research*** **8: 711-723, 1998.**

17. **Garret MR and Rapp JP. Two closely linked interactive blood pressure QTL on rat chromosome 5 defined using congenic Dahl rats.** ***Physiological Genomics*** **8: 81-86, 2001.**

18. **Jeffs B, Negrin CD, Graham D, Clark JS, Anderson NH, Gauguier D and Dominiczak AF. Applicability of a "speed" congenic strategy to dissect blood pressure quantitative trait loci on rat chromosome 2.** ***Hypertension*** **35: 179-187, 2000.**

19. **Lander ES and Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results.** ***Nat Genet*** **11: 241-247, 1995.**

20. **Moujahidine M, Dutil J, Hamet P and Deng AY. Congenic mapping of a blood pressure QTL on Chromosome 16 of Dahl rats.** ***Mammalian Genome*** **13: 153-156, 2002.**

21. **Moujahidine M, Lambert R, Dutil J, Palijan A, Sivo Z, Ariyarajah A and Deng AY. Combining congenic coverage with gene profiling in search of candidates for blood pressure QTLs in Dahl rats.** ***Hypertension Research*** **27: 203-212, 2004.**

22. **Palijan A, Dutil J and Deng AY. Quantitative trait loci with opposing blood pressure effects demonstrating epistasis on Dahl rat chromosome 3.** ***Physiological Genomics*** **15: 1-8, 2003.**

23. **Palijan A, lambert R, Sivo Z, Dutil J and Deng AY. Comprehensive congenic coverage revealing multiple BP QTLs on Dahl rat Chromosome 10.** ***Hypertension*** **42: 515-522, 2003.**

24. **Pravenec M, Gauguier D, Schott JJ, Buard J, Kren V, Bila V, Szpirer C, Szpirer J, Wang JM, Huang H and . Mapping of quantitative trait loci for blood pressure and cardiac mass in the rat by genome scanning of recombinant inbred strains.** ***J Clin Invest*** **96: 1973-1978, 1995.**

25. Saad Y, Garret MR and Rapp JP. Multiple blood pressure QTL on rat chromosome 1 defined by Dahl rat congenic strains. *Physiological Genomics* 4: 201-214, 2001.

26. Samani NJ, Gauguier D, Vincent M, Kaiser MA, Bihoreau MT, Lodwick D, Wallis R, Parent V, Kimber P, Rattray F, Thompson JR, Sassard J and Lathrop M. Analysis of quantitative trait loci for blood pressure on rat chromosomes 2 and 13. Age-related differences in effect. *Hypertension* 28: 1118-1122, 1996.

27. Schork NJ, Krieger JE, Trolliet MR, Franchini KG, Koike G, Krieger EM, Lander ES, Dzau VJ and Jacob HJ. A biometrical genome search in rats reveals the multigenic basis of blood pressure variation. *Genome Res* 5: 164-172, 1995.

28. Sivo Z, Malo B, Dutil J and Deng AY. Accelerated congenics for mapping two blood pressure quantitative trait loci on Chromosome 10 of Dahl rats. *J Hypertens* 20: 1-9, 2002.

29. Stoll M, Cowley AW, Jr., Tonellato PJ, Greene AS, Kaldunski ML, Roman RJ, Dumas P, Schork NJ, Wang Z and Jacob HJ. A genomic-systems biology map for cardiovascular function. *Science* 294: 1723-1726, 2001.

30. **Vincent M, Samani NJ, Gauguier D, Thompson JR, Lathrop GM and Sassard J. A pharmacogenetic approach to blood pressure in Lyon hypertensive rats. A chromosome 2 locus influences the response to a calcium antagonist. *J Clin Invest* 100: 2000-2006, 1997.**

Figure 1B. Fine mapping of C2QTL4. The linkage map is essentially the same as published previously, which is a composite map of Chr 2 (8). Solid bars under congenic strains symbolize the DSS chromosome fragments that have been replaced by that of the LEW rat. The entire region indicated by solid bars and junctions between the solid and open bars are homozygous for LEW, i.e. LL, on the map for all the markers listed in the corresponding positions. Open bars on ends of solid bars indicate the ambiguities of crossover breakpoints between markers. *Agtr1b*, angiotensin receptor type AT1B; *Prlr*, prolactin receptor. The rest of the markers are anonymous.

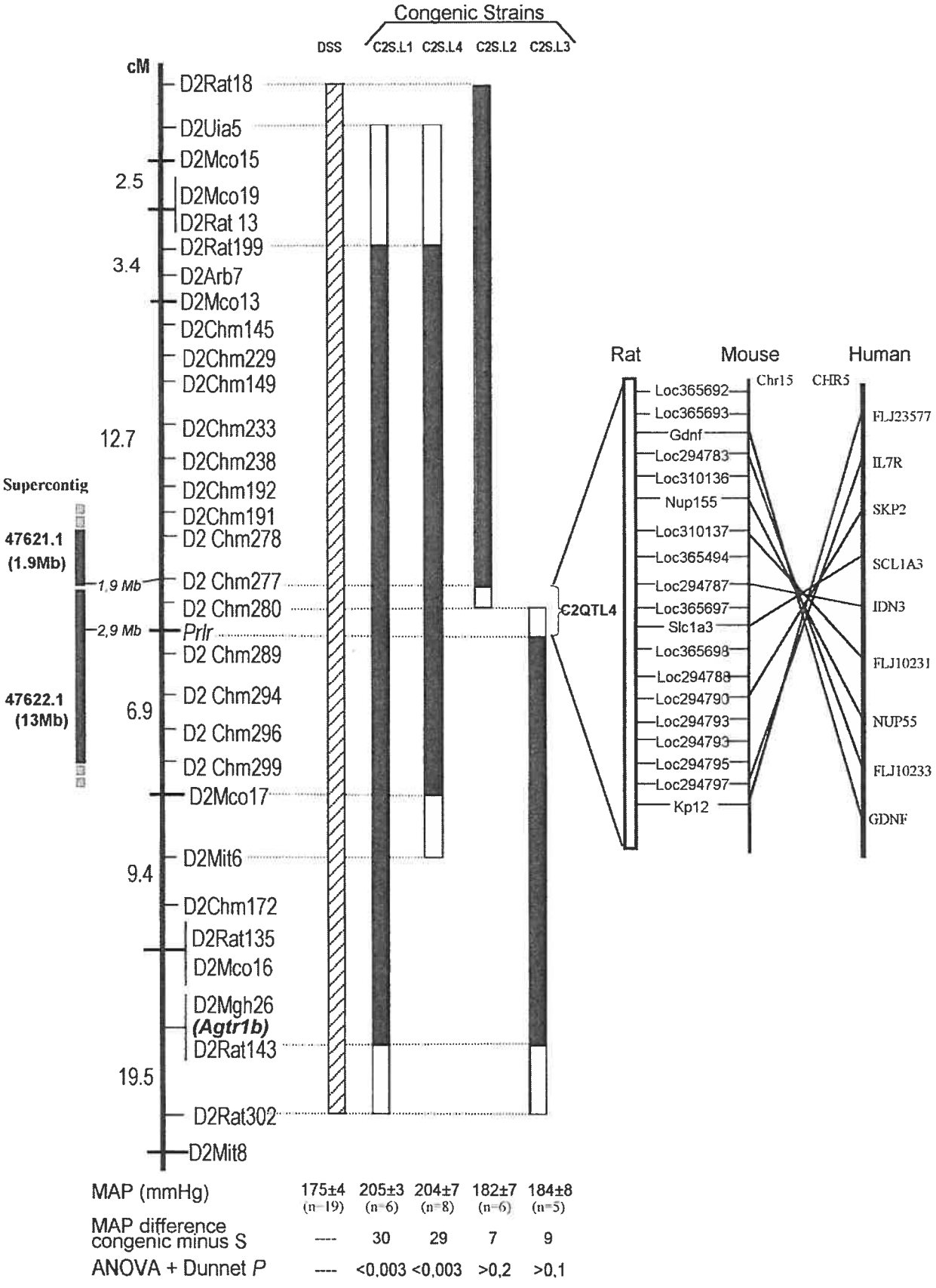
Primers for the new D2Chm markers are as follows: D2Chm145 (forward 5'ggggagagtttcaaccctactt 3', reverse 5'ggacccaatcggctcttaat 3'); D2Chm149 (forward 5' ttctgccttagtccccagtc 3', reverse 5' cctgcctaatttgcttttgg 3'); D2Chm172 (forward 5'gtggccagggactgagaata 3', reverse 5' cgtgaagagagggaacctca 3'); D2Chm175 (forward 5' ccagccactttgctgaagtt 3', reverse 5' ccccagctctggtaaacact 3'); D2Chm191(forward 5' tttccatgaatcctggcagt3', reverse 5' aaagccacatgccaattctc 3'); D2Chm192(forward 5' acagcagacattcgcaagc 3', reverse 5' agcagacacaaccctgagt 3'); D2Chm 229 (forward 5' aaagctgccacgaacaatct 3', reverse 5' tggacctaagtcccaaagga 3'); D2Chm233(forward 5' tgggtcccacctctagacac 3', reverse 5' ggccactttgtgctggataa 3'); D3Chm238(forward 5' ggatagccagggctacataga 3', reverse 5' cccatagggcccaatagttc3'); D2Chm277(forward 5' tcactggctcaaaagcctct 3', reverse 5' ggttgtaagttagaatactccccatc 3'); D2Chm278(forward 5' tctctgtctctgcccctacc 3', reverse 5' ctccaaaagagccgagtgtc3'); D2Chm280(forward 5' ccatagaaagatcacccttgc 3', reverse 5' tgggttcttactgacaaagatgc3'); D2Chm289(forward 5' cagcagaaatgcttgcctaa 3', reverse 5' tgacaagcaggaatagcctct 3'); D2Chm294(forward 5' ggcagaggcaggtgaatcta 3', reverse 5' tcacagagactatggcagactga 3'); D2Chm296(forward 5' tgtgcagccatggtacaaat 3', reverse 5' ccttttcacccttcccaaat 3'); D2Chm299(forward 5' accccagactcagcatttga 3', reverse 5' tcaaactaccccatcaggattc 3'). DSS, the Dahl salt-sensitive strain. Congenic strains were as follows: DSS.LEW-(D2Rat199-D2Rat143)/Lt (C2S.L1), DSS.LEW-(D2Rat18-D2Chm277)/Lt (C2S.L2), DSS.LEW-(*Prlr*-D2Rat143)/Lt (C2S.L3) and DSS.LEW-(D2Rat199-D2Mco17)/Lt (C2S.L4) respectively. MAP refers to the averaged mean arterial pressure during the period of measurement for each strain. ANOVA with the Dunnett's correction compares MAPs between DSS and

each of the congenic strains. The placement of C2QTL4 is to the right to congenic strains. Supercontigs were those taken from a database search at http://www.ncbi.nlm.nih.gov/mapview/. Numbers in parentheses below supercontigs represent their sizes in megabases (Mb). Genes found in the QTL-residing region of rat Chr 2, and homologous regions on mouse Chr15 and human CHR 5 are shown to the right of the map, which was based on a database search at http://www.ncbi.nlm.nih.gov/mapview/. They are as follows: *Gdnf*, glial cell line derived neurotrophic factor; *IDN3*, IDN3 protein; *IL7R*, interlukin 7 receptor; *Kpl2*, Kpl2 protein; *Nup155*, nucleoporin 155kDa; *SKP2*, S phase kinase associated protein 2; and *Slc1a3*, solute carrier family 1 member 3. Locs or FLJs refer to possible genes predicted by computer programs.

Figure 2B. Comparisons of BPs between congenic strains, and the DSS strain. a and d, systolic arterial pressures (DSAPs), b and e: diastolic arterial pressures (DAPs), and c and f, mean arterial pressures (SAPs). Each time point on the graph represents an average of 6-hour readings. Error bars represent SEM. n refers to the number of rats. Lew, the Lewis strain; DSS, Dahl salt-sensitive strain. For designations of congenic strains, see the legend for Figure 1.

Figure 3B. Comparisons of QTLs localized on Chr 2. The rat map is synthesized from several sources including the references cited and http://www-genome.wi.mit.edu; http://rgd.mcw.edu; http://www.broad.mit.edu/resources.html; http://www.well.ox.ac.uk/~bihoreau/; http://ratmap.ims.u-tokyo.ac.jp. Markers in parentheses are adopted from the above sources. The position of C2QTL4 fine mapped in the current work is as defined by comparative congenics from Figure 1 and is indicated by a dark bar. Comparisons of BP QTL localizations on Chr 2 reported in the literature are shown to the right of the map. Shaded bars to the right of the map indicate QTL interval estimates, to the best of our knowledge, for BP QTL regions localized in separate studies specified by reference numbers situated directly above.

Figure 1


Congenic Strains
DSS
C2S.L1
C2S.L4
C2S.L2
C2S.L3
cM
D2Rat18
D2Uia5
D2Mco15
2.5
D2Mco19
D2Rat 13
D2Rat199
3.4
D2Arb7
D2Mco13
D2Chm145
D2Chm229
D2Chm149
12.7
D2Chm233
D2Chm238
D2Chm192
D2Chm191
D2 Chm278
D2 Chm277
D2 Chm280
Prlr
D2 Chm289
6.9
D2 Chm294
D2 Chm296
D2 Chm299
D2Mco17
9.4
D2Mit6
D2Chm172
D2Rat135
D2Mco16
D2Mgh26
(Agtr1b)
D2Rat143
19.5
D2Rat302
D2Mit8
Supercontig
47621.1
(1.9Mb)
1,9 Mb
2,9 Mb
47622.1
(13Mb)
C2QTL4
Rat
Mouse
Human
Loc365692
Loc365693
Gdnf
Loc294783
Loc310136
Nup155
Loc310137
Loc365494
Loc294787
Loc365697
Slc1a3
Loc365698
Loc294788
Loc294790
Loc294793
Loc294793
Loc294795
Loc294797
Kp12
Chr15
CHR5
FLJ23577
IL7R
SKP2
SCL1A3
IDN3
FLJ10231
NUP55
FLJ10233
GDNF
MAP (mmHg)
175±4 (n=19)
205±3 (n=6)
204±7 (n=8)
182±7 (n=6)
184±8 (n=5)
MAP difference congenic minus S

30
29
7
9
ANOVA + Dunnet P

<0,003
<0,003
>0,2
>0,1

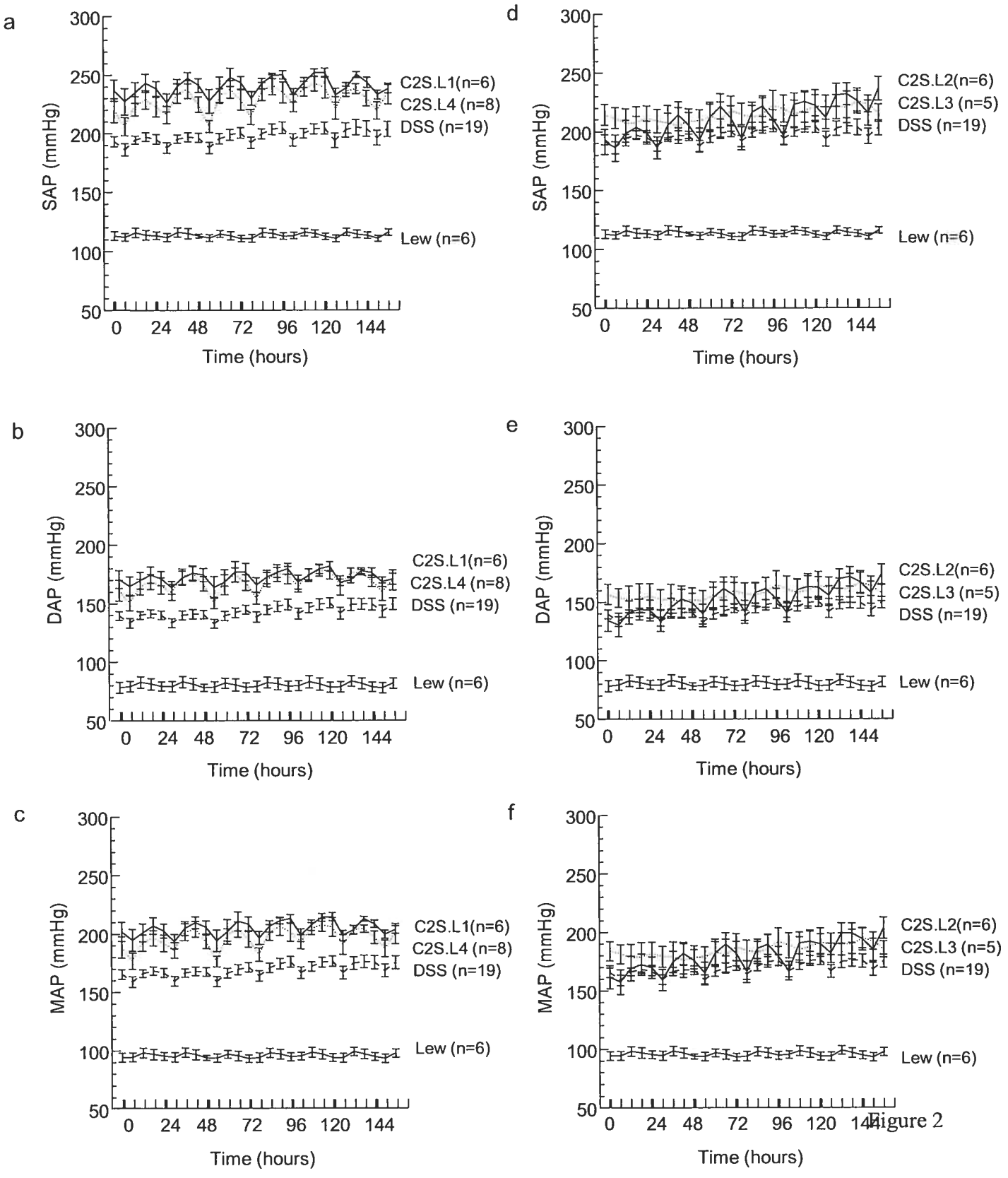
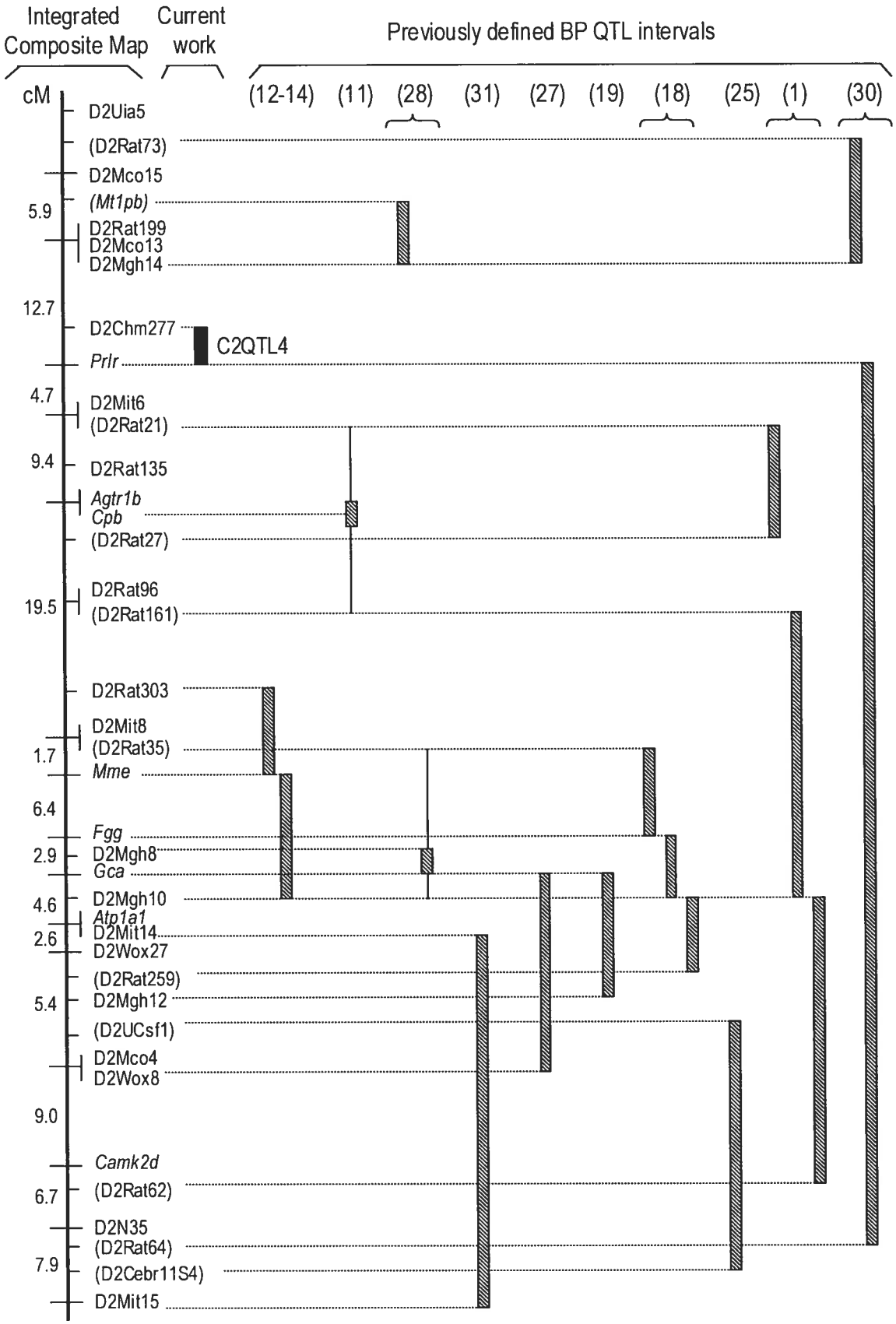

a
SAP (mmHg)
300
250
200
150
100
50
0 24 48 72 96 120 144
Time (hours)
C2S.L1(n=6)
C2S.L4 (n=8)
DSS (n=19)
Lew (n=6)
d
SAP (mmHg)
Time (hours)
C2S.L2(n=6)
C2S.L3 (n=5)
DSS (n=19)
Lew (n=6)
b
DAP (mmHg)
Time (hours)
C2S.L1(n=6)
C2S.L4 (n=8)
DSS (n=19)
Lew (n=6)
e
DAP (mmHg)
Time (hours)
C2S.L2(n=6)
C2S.L3 (n=5)
DSS (n=19)
Lew (n=6)
c
MAP (mmHg)
Time (hours)
C2S.L1(n=6)
C2S.L4 (n=8)
DSS (n=19)
Lew (n=6)
f
MAP (mmHg)
Time (hours)
C2S.L2(n=6)
C2S.L3 (n=5)
DSS (n=19)
Lew (n=6)

Figure 2


Integrated Composite Map
Current work
Previously defined BP QTL intervals
(12-14)
(11)
(28)
(31)
(27)
(19)
(18)
(25)
(1)
(30)
cM
D2Uia5
(D2Rat73)
D2Mco15
5.9
(Mt1pb)
D2Rat199
D2Mco13
D2Mgh14
12.7
D2Chm277
C2QTL4
Prlr
4.7
D2Mit6
(D2Rat21)
9.4
D2Rat135
Agtr1b
Cpb
(D2Rat27)
19.5
D2Rat96
(D2Rat161)
D2Rat303
D2Mit8
1.7
(D2Rat35)
Mme
6.4
Fgg
2.9
D2Mgh8
Gca
4.6
D2Mgh10
Atp1a1
2.6
D2Mit14
D2Wox27
(D2Rat259)
5.4
D2Mgh12
(D2UCsf1)
D2Mco4
D2Wox8
9.0
Camk2d
6.7
(D2Rat62)
D2N35
(D2Rat64)
7.9
(D2Cebr11S4)
D2Mit15

Discussion

Chapter 8

Discussion

The influence of genetic and environmental factors

8.1 Environmental factors

Every step of our strategy to localise a QTL has factors that can limit the validity of the results. For example, such factors as age, sex and diet can vary the results which are obtained and make us question the validity of the study and the isolation of the QTL. These environmental factors must then be taken into account when considering or analysing the results.

8.1.1 Sex of the animal

The detection of different QTLs for blood pressure in males and females shows us that there exists certain effects specific to sex and the susceptibility to hypertension. (74,75) In female rats, hypertension appears at a later time and progresses at a more moderate pace than in males. Many female hormones, such as oestrogen, can interfere with the control of blood pressure. On the other hand, the difference observed in blood pressure between males and females cannot be explained uniquely by hormones and must imply a completely different group of regulatory genes.

Most genetic linkage analyses for blood pressure have been done using male rats. Male rats develop hypertension much more quickly than females and due to their faster growth, the measure of arterial pressure can be done at a younger age as well. This is why the majority of studies done with the goal of isolating the QTLs for arterial blood pressure have not evaluated the effect of sex on the expression of the QTLs observed.

QTLS common to both females and males do exist, but the presence of QTLs specific to sex creates a need for specific studies with females and the need for specific female intervention in the human.

8.1.2 Age of the animal

Most genetic linkage analysis studies have been done in adult animals because arterial pressure augments with age. Development is finished and the phenotype is expressed entirely only at adult age. Telemetry analysis is very rigorous when looking at animal age; starting at ten weeks of age the arterial pressure is evaluated (See figure 11)

8.1.3 Animal diet

The rats which were used in this study have been fed a high salt diet. It has been demonstrated that diet can vary arterial pressure in the S rat (76). Salt intake is based on food intake; therefore it is possible that certain rats eat more than others. To counter this variable effect, the weight of the rats used in telemetry is situated in a precise interval. (77,78) But, one must note that it is possible that certain rats experience more weight gain with less food, especially the S and congenic strains. It is therefore very important to consider that hypertension can be related to weight control (79) such as in the genetically hypertensive SHR rats (80).

8.1.4 Method of measure of arterial pressure

Three methods are used to measure arterial pressure in the rat: the fastening of the tail, telemetry and the arterial cathete (81). The method using the tightening of the tail measures the systolic arterial pressure and implies the mobilisation of the extremity of the rat tail. All the hypertensive model strains have been selected using this method, including the original S rat (82), the R rat as well as the SHR rat. It is a method which is fast and cost efficient which permits to take many measures at a larger scale.

On the other hand, the measures are indirect, imprecise, last only minutes and do not detect blood pressure variation under 10mmHg (83).Moreover, this method causes stress to an animal, due to its physical immobilization and this in turn can vary the arterial pressure. Lastly, it is important to note that it is important to be constant in the measuring period because arterial pressure can vary from hour to hour.

Telemetry is a method which is reliable and precise. It is direct, continuous and measures long term arterial pressure without interruption. The fact that we measure on a 24 h timeframe permits us to take into account the diurnal variations of the arterial pressure. The only downfall is that it is costly and invasive because it requires a surgical implant.

8.2 Genetic factors

The genetic background composition must be considered as a factor that may influence blood pressure results. The normotensive strain used in F2 population studies can have an important effect. For example, a genome scan study using F2 populations derived from crosses such as S x R, S x BN, S x WKY and S x MNS detected QTLs on many chromosomes.(84) Some of these QTLs were observed only in one of the four crosses, this demonstrates the effect of the genetic background on the expression of the phenotype. The use of many normotensive strains permits us to introduce various alleles and genetic backgrounds at the QTL site. On the other hand, the genetic background of a congenic strain is important to observe the effect of a complex trait.

Another problem of the genetic background is the possibility of the presence of residual loci which originate from the donor strain. These loci would have gone unnoticed during the verification of the genetic background which is done using polymorphic markers which are distributed throughout the genome. These loci may interact with the QTL and influence arterial pressure.

8.2.2 The position effect

The function of a gene does not solely depend on the gene itself because it is influenced by other genes or DNA sequences which are situated close to the gene (83). A sequence situated close to a gene can activate or repress the promoter gene. This sequence might find itself close to the gene due to a chromosomal rearrangement during mitosis or meiosis.

It is important to consider these background effects because they may influence results. In the long run, it is difficult to determine if an effect on arterial pressure is due to a substituted chromosomal fragment in a congenic strain or simply due to a diffused genetic background effect. The strategy used to evaluate if a position effect is responsible for arterial pressure variation is to construct a congenic strain which possesses a substituted chromosomal fragment right next to, but without overlapping, the previous strain (84).

8.2.3 Interaction between the QTLs

Presently many blood pressure QTLs have been localised using S rats (85). An interesting aspect that emerges from these studies is that it is possible to consider blood pressure, a polygenic trait, and to fraction it into multiple monogenic traits. In other words, every blood pressure QTL could function as a unique genetic unit independent of other QTLs in an appropriate genetic background.

Genetic linkage analysis studies demonstrate the presence of a QTL is most often in very large chromosomal fragments. With the help of congenics, it is possible to determine how many QTLs are situated in a candidate region as well as evaluating their individual and combined effect on blood pressure. For example, on chromosome 10 of the S rat, it was determined that a chromosomal fragment which was found by genetic linkage analysis contained 3 isolated QTLs which acted in an additive fashion (86).

Another example would be chromosome 3 where an epistasis has been demonstrated to occur. Two QTLs which have an opposite effect on blood pressure have been localised on chromomose 3 using many congenic strains. When these 2 QTLs are present in the same congenic strain, the effect of the blood pressure augmenting QTL is masked by that of the blood pressure lowering QTL (87). The fact that LEW contains alleles that can increase blood pressure is surprising. Similar results have also been found on chromosome 8 as well as in this study on chromosome 2. These results demonstrate that to be normotensive, a strain such as LEW is not obliged to be carrying blood pressure lowering alleles in every one of it's QTLs. Therefore, the determination of blood pressure in rats would be the result of a refined structure of QTLs, sometimes possessing contrary effects, and would form a genetic organisation which could be applied to other mammals such as the human.

Chr	Contrasting strains	Number of QTLs	Confirmed by congenic strains	Details
1	LEW	3	Yes	
2	WKY	3	Yes	Interaction with QTLs of Chr.10 (62)
	MNS	3	Yes	
	LEW	1	Yes	
3	R	1	Yes	
	LEW	2	Yes	2 QTLs=epistasis (87)
5	LEW	2	Yes	
7	R	1	Yes	
8	LEW	2	Yes	Two QTLs with opposing effcets on blood pressure (71)
9	R	1	Yes	
10	MNS	2	Yes	
	LEW	3	Yes	Three additive QTLs (59)
12	WKY	1	No	
13	R	1	Yes	
	BN	1	Yes	
15	WKY	1	No	
16	LEW	1	Yes	
	BN	1	Yes	
17	LEW	1	Yes	
18	LEW	2	Yes	
	BN	1	Yes	

Table IV- BP QTLs localized in the Dahl *salt-sensitive* rat

Adapted from Deng A.Y (55). The contrasting strain is the normotensive rat strain implied in localizing the QTL on the chromosome. Chr, chromosome; S, Dahl *salt-sensitive*; LEW, Lewis; WKY, Wistar-Kyoto; MNS, Milan *normotensive*; R, Dahl *salt-resistant*; BN, Brown-Norway.

At first glance once can assume that the effects of multiple QTLs are additive. But in a complex disease such as hypertension, there exist epistatic interactions between QTLs (88). An epistasis is a dominance of one gene over another. This interaction is inevitable when we observe the number of different blood pressure QTLs which have been isolated throughout the genome and their drastic effect when evaluated separately. Therefore, these QTLs interact amongst themselves and do not all equally contribute to the overall blood pressure, this means the effect of one QTL can mask that of another (89).

Chapter 9
Discussion of present work

Hypertension is a complex disease where many environmental and genetic factors contribute to a variation in blood pressure. There are two forms of this disease: essential and monogenic. The identification of causative genes involved in hypertension will permit a better comprehension of the pathophysiology of the disease as well as a better treatment. However, the essential form is multigenic, this renders its genetic dissection quite difficult. Using animal models facilitates this task.

It was previously suggested that the region between D2rat 18 and Prlr markers on chromosome 2 is linked to essential hypertension. We wanted to verify this hypothesis by determining the presence of a QTL in this region. By developing a detailed chromosomal map we were able to construct congenic strains using the rat as a model. This permitted us to demonstrate that the considered region has a significant impact on arterial pressure caused by a blood pressure raising QTL.

There are many findings in this work, but the essential ones to be retained are the following four. The first is that LEW as well as Dahl *Salt-Sensitive* can carry blood pressure raising alleles even thought this strain is thought to be normotensive and not hypertensive. This fact indicates that it is not a single QTL, but rather it is how this QTL interacts with others, that determine the overall BP of a strain. Among the mechanisms of interactions, one can be epistasis.

Also, in the current work we have succeeded in reducing the area of interest of the QTL to an interval of around 3 cM, we were able to accomplish this through much breeding and by using comparative genomics. By comparing the two strains that had no blood pressure effect with those that did we were able to determine that this region contained 19 possible genes, 4 known and 15 undefined Locs (Table V).

Gene	Name	Homology	Alias
LOC365692	similar to 40S ribosomal protein S9		
LOC365693	similar to hypothetical protein		
Gdnf	glial cell line derived neurotrophic factor	H: GDNF M: Gdnf	
LOC294783			
LOC310136	moved to Chr5		
Nup155	nucleoporin 155	H: NUP155 M:Nup155	
LOC310327	predicted similar to hypothetical protein FLJ13231 (predicted)	H:FLJ13231 M:2410089E03	RGD1310081
LOC365494			
LOC294787			
LOC365697	similar to ribosomal protein S12		
FLC183	no longer exists		
LOC365698			
LOC294788			
LOC294790	predicted similar to S-phase kinase-associated protein 2 (F-box protein Skp2) (F-box/WD-40 protein 1) (predicted)		RGD1562456
LOC294793	predicted similar to Hypothetical protein MGC37820 (predicted)	H:UGT3A2 M:Ugt3a1 and Ugt3a2 (UDP glycosyltransferase 3 family, polypeptide A2 or A1)	RGD1564365
LOC294795	predicted calcyphosine-like (predicted)	H:CAPSL M:Capsl	Capsl; RGD1308776
LOC294797			
KP12	no longer exists		

Table V- Table of genes and Locs in QTL region
Includes their homology in the human and rat as well as their alias

Of the 19 genes included in the QTL region, 7 Locs have no defined functions which have been discovered as of yet. This leads us to believe that the novel gene which would influence blood pressure would be one of these because the other Locs which have defined functions are not thought to be linked to hypertension. The other four known genes are also not thought to be linked to hypertension and with the updated version of the genetic sequences two of the four genes do not even exist in this region anymore. Of the two genes that still exist, Gdnf is known to be essential to brain function and the second Nup155 is important to the pores of cellular membranes.

In this study we have confirmed the presence of a QTL but at the same time *Agtr1b* (*Angiotensin Receptor 1B)*, which has been thought to be a candidate gene in the past has been disqualified because C2S.L3 had a BP not distinguishable from that of Dahl *Salt-Sensitive*. Besides *Agtr1b* there seem to be no apparent genes which are of interest at the moment but with further studies and more precise QTL mapping it will lead to the identification of a novel gene previously unknown to influence BP.

In future studies, in order for one to truly prove that this region does indeed contain a blood pressure QTL, we must make a congenic strain that specifically contains the interval of D2Chm145 and Prlr. This small strain which should measure at the most 1cM would be ideal not only for confirmation of this QTL but also for final molecular identification. Another advantage a smaller and more specific congenic strain would bring is finding out whether or not the fragment between D2Chm277 and *Prlr* contains one or two QTLs. We must remember that the possibility of there being two QTLs in this region cannot be excluded.

We also concluded that for the moment *Agtr1b* is not supported as a candidate gene for the QTL because congenic strain C2S.L3 harbors it, and yet, did not show a BP effect (Figures 1 and 2 in article). Using homology mapping with the rat, mouse and human genomes, we found no obvious candidate genes which have been known to

influence blood pressure or hypertension in the region containing the QTL. Thus, it is most likely that this QTL will represent a novel gene for BP regulation.

C2QTL4 identified in our current work seems to be unique in its chromosome location and, in contrast to other BP QTLs, in its BP-increasing effect from Dahl *Salt-Sensitive*. These effects have to be further studied in order to narrow and target the region further in order to find a candidate gene and to establish a mechanism by which this QTL interacts with others.

Conclusion

Chapter 10
Conclusion and Perspectives

A large part of my work in the laboratory consisted in generating a genetic map rich and dense in markers for chromosome 2. This was accomplished by integrating the maps available on the internet, by constructing new markers from the genomic sequence which is presently available and by positioning them on congenic strains.

A good genetic map permits a better characterization of the congenic strains and substrains. This in turn permits a better detection of possible cross-overs during breeding. The rats presenting these interesting traits are used to develop congenic sub-strains. This step is crucially important when the QTL regions are small.

Another part of my work consisted of genotyping rats each week. The genotyping was done with PCR by using different polymorphic markers and positioning them on maps. The genotyping of rats is very important and demands much precision.

Given the number of candidate genes in every QTL region, the next step would be to reduce these QTL regions. This can be accomplished with the construction of new congenic sub-strains. Telemetry will then be used to identify the positive and negative strains therefore reducing the QTL region. Once the region is reduced to 1 cM comparative sequencing can then begin searching for mutations.

The application of computer and internet tools which are available have played a big role in this project. With the advancement of technology as well as that availability of the genomic sequence more and more tools are at hand. One can only wonder what the future holds for a science which is ever evolving.

Reference List

1. Campbell, N. A. Circulation et échanges gazeux. *In* R. Mathieu (ed.), Biologie, 3 ed, pp. 820-828. St-Laurent: Éditions du Renouveau Pédagogique Inc., 1995.

2. Lewicki, J. A. and Preotter, A. A. Physiological studies of the natriuretic peptide family. *In* J. H. Laragh and B. M. Brenner (eds.), Hypertension: pathology, diagnosis and management, pp. 1029-1053. New York: Raven Press Ltd, 1995.

3. Kimura, G. and Brenner, B. M. The renal basis for salt sensitivity in hypertension. *In* J. H. Laragh and B. M. Brenner (eds.), Hypertension: pathophysiology, diagnosis and management, 2 ed, pp. 1569-1605. New York: Raven Press Ltd, 1995.

4. Robertson, J. I. S. Renin and angiotensin: a history review. The Renin-Angiotensin System, Robertson JIS, Nicholls MG ed, pp. 1-18. London: Gower Medicas Publishing, 1993.

5 Silverthorn, D. U. Human Physiology an Integrated approach. Dinsey, H. Thir

Edition. 2003. New York, Benjamin-Cummings Publishing Company.

6. Iwai, N. and Inagami, T. Identification of two subtypes in the rat type I angiotensin II receptor. FEBS Lett., *298*: 257-260, 1992.

7. Sasamura, H., Hein, L., Krieger, J. E., and et al. Cloning, characterization, and expression of two angiotensin receptors (AT-1) isoforms from the mouse genome. Biochem.Biophys.Res.Commun., *185*: 253-259, 1992.

8. Kakar.S.S., Sellers, J. c., Devor, D. C., Musgrove, L. C., and Neill, J. S. Angiotensin II type-1 receptor subtype cDNAs:differential tissue expression and hormonal regulation. Biochem.Biophys.Res.Commun., *183*: 1090-1096, 1992.

9. Vasan, R. S., Beiser, A., Seshadri, S., Larson, M. G., Kannel, W. B., D'Agostino, R. B., and Levy, D. Residual lifetime risk for developing hypertension in middle-aged women and men: The Framingham Heart Study. JAMA, *287*: 1003-1010, 2002.

10. The sixth report of the joint national committee on the prevention, detection, evaluation and treatment of high blood pressure. Arch.Intern.Med., *157*: 2413-2446, 1997.

11. Kannel, W. B. Elevated systolic blood pressure as a cardiovascular risk factor. Am.J.Cardiol., *85*: 251-255, 2000.

12. Mosterd, A., D'Agostino, R. B., Silbershatz, H., Sytkowski, P. A., Kannel, W. B., Grobbee, D. E., and Levy, D. Trends in the prevalence of hypertension, antihypertensive therapy, and left ventricular hypertrophy from 1950 to 1989. N.Engl.J.Med, *340*: 1221-1227, 1999.

13. Tanira M, Balushi KA. Genetic Variations related to hypertension: a review. *Journal of Human Hypertension* 2004;Advance Online Publication:1-13.

14. Hamet P, Pausova Z, Adarichev V, Adaricheva K, Tremblay J. Hypertension: genes and environment. *Journal of Hypertension* 1998;16:397-418.

15. Hansson, L., Kilander, L., and Ohrvall, M. Hypertension: A companion to Benner and Rector's *The Kidney*. Oparil, S. and Weber, M. A. 6th, 4-20. 2000. Philadelphia, W.B.Saunders Company.

16.Rapp JP. Genetic Analysis of Inherited Hypertension in the Rat. *Physiological Reviews* 2000;80:135-72.

17. Spiegelman, D., Israel, R. G., Bouchard, C., and Willett, W. C. Absolute fat mass, percent body fat, and body-fat distribution which is the real determinant of blood pressure and serum glucose. Am.J.Clin.Nutr., *55*: 1033-1044, 1992.

18.Whyte, H. M. Blood pressure and obesity. Circulation, *19*: 511-516, 1956.

19. Flack, J. M. and Staffileno, B. A. Hypertension: A Companion to Benner and Rector's The Kidney. Oparil, S. and Weber, M. A. 6th, 558-563. 2000. Philadelphia, W.B. Saunders Company.

20. Page, L. B., Damon, A., and Moellering, R. C. Antecedents of cardiovascular disease in six Solomon islands societies. Circulation, *49*: 1132-1146, 1974.

21. Weinberger, M. H. Salt sensitivity: does it play an important role in the pathogenesis and treatment of hypertension? Curr.Opin.Nephrol.Hypertens., *5*: 205-208, 1996.

22. Iwamoto, T., Kita, S., Zhang, J., Blaustein, M. P., Arai, Y., Yoshida, S., Wakimoto, K., Komuro, I., and Katsuragi, T. Salt-sensitive hypertension is triggered by Ca2+ entry via Na+/Ca2+ exchanger type-1 in vascular smooth muscle. Nat.Med., *10*: 1193-1199, 2004.

23. Rapp JP. Genetic Analysis of Inherited Hypertension in the Rat. *Physiological Reviews* 2000;80:135-72.

24.Cowley AW, Stoll M, Greene AS, Kaldunsky ML, Roman RJ, Tonellato PJ et al. Genetically defined risk of salt sensitivity in an intercross of Brown Norway and Dahl S rats. *Physiological Genomics* 2000;2:107-15

25. Cowley AW, Stoll M, Greene AS, Kaldunsky ML, Roman RJ, Tonellato PJ et al. Genetically defined risk of salt sensitivity in an intercross of Brown Norway and Dahl S rats. *Physiological Genomics* 2000;2:107-15

26. Frankel WN, Schork NJ. Who's afraid of epistasis. *Nature Genetics* 1996;14.

27. Glazier AM, Nadeau JH, Aitman TJ. Finding Genes That Underlie Complex Traits. *Science* 2002;2345-9.

28. Winslow RL, Boguski MS. Genome Informatics. *Circulation Research* 2003;92:953-6129

29. Rapp JP, Garrett MR, Deng AY. Construction of a double congenic strain to prove an Epistatic Interaction on Blood Pressure between Rat Chromosome 2 and 10. *Journal of Clinical Investigation* 1998;101:1591-5.

30. Veerasingham SJ, Sellers KW, Raizada MK. Functional genomics as an emerging strategy for the investigation of central mechanisms in experimental hypertension. *Biophysics and Molecular Biology* 2004;84:107-23.

31. Lifton RP, Gharavi AG, Geller DS. Molecular Mechanism of Human Hypertension. *Cell* 2001;104:545-56.

32. Glazier AM, Nadeau JH, Aitman TJ. Finding Genes That Underlie Complex Traits. *Science* 2002;2345-9.

33. Clerget-Darpoux F. Overview of strategies for complex genetic diseases. *Kidney International* 1998;53:1445.

34. Deng, A. Y., Smith-Mensah, W. H., Hoebee, B., Garrett, M., and Rapp, J. P. Linkage mapping of rat chromosome markers generated from chromosome-sorted DNA. Mamm.Genome, *9*: 38-43, 1998.

35. Deng, A. Y., Smith-Mensah, W. H., Hoebee, B., Garrett, M., and Rapp, J. P. Linkage mapping of rat chromosome markers generated from chromosome-sorted DNA. Mamm.Genome, *9*: 38-43, 1998.

36. Hamet, P., Pausova, Z., Tremblay, J., and Deng, A. Y. Hypertension as a genetic desease. *In* G. Mancia, J. Chalmers, S. Julius, T. Saruta, M. Weber, A. Ferrari, and I. Wilkinson (eds.), Manual of hypertension, pp. 49-75. London: Harcourt Publishers Limited, 2002.

37. Rapp, J. P. Genetic analysis of inherited hypertension in the rat. Physiol.Rev, *80*: 135-172, 2000.

38. Kato, N. Genetic analysis in human hypertension. Hypertens.Res., *25*: 319-327, 2002.

39. Rubattu, S., Struk, B., Kreutz, R., Volpe, M., and Lindpaintner, K. Animal models of genetic hypertension: what can we learn for human hypertension? Clin.Exp.Pharmacol.Physiol., *Suppl.2*: S386-S393, 1995.

40. Lee WK. Genetics of Hypertension: from experimental models to clinical applications. *Journal of Human Hypertension* 2004;14:631-47.

41. Garrett MR, Dene H, Walder R, Zhang Q-Y, Cicila GT, Assadnia S et al. Genome Scan and Congenic Strains for Blood Pressure QTL Using Dahl Salt-Sensitive Rats. *Genome Research* 1998;8:711-23

42. Hedrich, H. J. History,Strains, and Models in the Laboratory Rat, Krinke,G.J. ed.San Diego: Academic, 2000.

43. Gibbs, R. A., Weinstock, G. M., Metzker, M. L., Muzny, D. M., Sodergren, E. J., Scherer, S., Scott, G., Steffen, D., Worley, K. C., Burch, P. E., Okwuonu, G., Hines, S., Lewis, L., DeRamo, C., Delgado, O., Dugan-Rocha, S., Miner, G., Morgan, M., Hawes, A., Gill, R., Celera, Holt, R. A., Adams, M. D., Amanatides, P. G., Baden-Tillson, H., Barnstead, M., Chin, S., Evans, C. A., Ferriera, S., Fosler, C., Glodek, A., Gu, Z., Jennings, D., Kraft, C. L., Nguyen, T., Pfannkoch, C. M., Sitter, C., Sutton, G. G., Venter, J. C., Woodage, T., Smith, D., Lee, H. M., Gustafson, E., Cahill, P., Kana, A., Doucette-Stamm, L., Weinstock, K., Fechtel, K., Weiss, R. B., Dunn, D. M., Green, E. D., Blakesley, R. W., Bouffard, G. G., De Jong, P. J., Osoegawa, K., Zhu, B., Marra, M., Schein, J., Bosdet, I., Fjell, C., Jones, S., Krzywinski, M., Mathewson, C., Siddiqui, A., Wye, N., McPherson, J., Zhao, S., Fraser, C. M., Shetty, J., Shatsman, S., Geer, K., Chen, Y., Abramzon, S., Nierman, W. C., Havlak, P. H., Chen, R., Durbin, K. J., Egan, A., Ren, Y., Song, X. Z., Li, B., Liu, Y., Qin, X., Cawley, S., Worley, K. C., Cooney, A. J., D'Souza, L. M., Martin, K., Wu, J. Q., Gonzalez-Garay, M. L., Jackson, A. R., Kalafus, K. J., McLeod, M. P., Milosavljevic, A., Virk, D., Volkov, A., Wheeler, D. A., Zhang, Z., Bailey, J. A., Eichler, E. E., Tuzun, E., Birney, E., Mongin, E., Ureta-Vidal, A., Woodwark, C., Zdobnov, E., Bork, P., Suyama, M., Torrents, D., Alexandersson, M., Trask, B. J., Young, J. M., Huang, H., Wang, H., Xing, H., Daniels, S., Gietzen, D., Schmidt, J., Stevens, K., Vitt, U., Wingrove, J., Camara, F., Mar, A. M., Abril, J. F., Guigo, R., Smit, A., Dubchak, I., Rubin, E. M., Couronne, O., Poliakov, A., Hubner, N., Ganten, D., Goesele, C., Hummel, O., Kreitler, T., Lee, Y. A., Monti, J., Schulz, H., Zimdahl, H., Himmelbauer, H., Lehrach, H., Jacob, H. J., Bromberg, S., Gullings-Handley, J., Jensen-Seaman, M. I., Kwitek, A. E., Lazar, J., Pasko, D., Tonellato, P. J., Twigger, S., Ponting, C. P., Duarte, J. M., Rice, S., Goodstadt, L., Beatson, S. A., Emes, R. D., Winter, E. E., Webber, C., Brandt, P., Nyakatura, G., Adetobi, M., Chiaromonte, F., Elnitski, L., Eswara, P., Hardison, R. C., Hou, M., Kolbe, D., Makova, K., Miller, W., Nekrutenko, A., Riemer, C., Schwartz, S., Taylor, J., Yang, S., Zhang, Y., Lindpaintner, K., Andrews, T. D., Caccamo, M., Clamp, M., Clarke, L., Curwen, V., Durbin, R., Eyras,

E., Searle, S. M., Cooper, G. M., Batzoglou, S., Brudno, M., Sidow, A., Stone, E. A., Venter, J. C., Payseur, B. A., Bourque, G., Lopez-Otin, C., Puente, X. S., Chakrabarti, K., Chatterji, S., Dewey, C., Pachter, L., Bray, N., Yap, V. B., Caspi, A., Tesler, G., Pevzner, P. A., Haussler, D., Roskin, K. M., Baertsch, R., Clawson, H., Furey, T. S., Hinrichs, A. S., Karolchik, D., Kent, W. J., Rosenbloom, K. R., Trumbower, H., Weirauch, M., Cooper, D. N., Stenson, P. D., Ma, B., Brent, M., Arumugam, M., Shteynberg, D., Copley, R. R., Taylor, M. S., Riethman, H., Mudunuri, U., Peterson, J., Guyer, M., Felsenfeld, A., Old, S., Mockrin, S., and Collins, F. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. Nature, *428*: 493-521, 2004.

44. Lee WK. Genetics of Hypertension: from experimental models to clinical applications. *Journal of Human Hypertension* 2004;14:631-47.

45. Rat Genome Sequencing Project Consortium. Genome sequencec of the Brown Norway rat yields insights into the mammilian evolution. *Nature* 2004;428:493-521.

46. Garrett MR, Dene H, Walder R, Zhang Q-Y, Cicila GT, Assadnia S et al. Genome Scan and Congenic Strains for Blood Pressure QTL Using Dahl Salt-Sensitive Rats. *Genome Research* 1998;8:711-23.

47. Rapp JP. Genetic Analysis of Inherited Hypertension in the Rat. *Physiological Reviews* 2000;80:135-72.

48. McBride MW, Charhcar FJ, Graham D, Miller WH, Strahorn P, Carr FJ et al. Functional genomics in rodent models for hypertension. *Journal of Physiology* 2003;554:56-63.

49. Deng AY, Smith-Mensah WH, Hoebee B, Garrett MR, Rapp JP. Linkage mapping of rat chromosome markers generated from chromosome-sorted DNA. *Mammalian Genome* 1998;9:38-43

50. Lee WK. Genetics of Hypertension: from experimental models to clinical applications. *Journal of Human Hypertension* 2004;14:631-47.

51. Stec DE, Davisson RL, Sigmund CD. Transgenesis and Gene Targeting in the Mouse Tools for studying Genetic Determinants of Hypertension. *Trends in Cardiovascular Medecine* 1998;8:256-

52. Sivo Z, Malo B, Dutil J, Deng AY. Accelerated congenics for mapping two blood pressure quantitative trait loci on chromosome 10 of Dahl Rats. *Journal of Hypertension* 2002;20:45-53.

53. McBride MW, Charhcar FJ, Graham D, Miller WH, Strahorn P, Carr FJ et al. Functional genomics in rodent models for hypertension. *Journal of Physiology* 2003;554:56-63.

54. Veerasingham SJ, Sellers KW, Raizada MK. Functional genomics as an emerging strategy for the investigation of central mechanisms in experimental hypertension. *Biophysics and Molecular Biology* 2004;84:107-23.

55. Deng AY. Molecualr analysis of the rat Inducible Nitric Oxide Synthase Gene. *Clinical and Experimental Hypertension* 1998;20:53-65.

56. Hubner N, Lee Y-A, Lindpaintner K, Ganten D, Kreutz R. Congenic Substitution Mapping excludes SA as a Candidate Gene Locus for a Blood Pressure Quantitative Trait Locus on Rat Chromosome 1. *Hypertension* 1999;34:643-8.

57. Monti J, Zimdahl H, Schulz H, Plehm R, Ganten D, Hubner N. The role of Wnk4 in Polygenic Hypertension, A candidate gene Analysis on Rat Chromosome 10. *Hypertension* 2003;41:938-42.

58. St.Lezin E, Liu W, Wang J-M, Yang Y, Qi N, Kren V et al. Genetic Analysis of Rat Chromosome 1 and the Sa Gene in Spontaneous Hypertension. *Hypertension* 2000;35, part 2:225-30.

59. Garrett MR. Two linked blood pressure quantitative trait loci on chromosome 10 by Dahl Rat Congenic Strains. *Hypertension* 2004;38:779-85.

60. Saad Y, Garrett MR, Rapp JP. Multiple blood pressure QTL on rat chromosome 1 defined by Dahl rat congenic strains. *Physiological Genomics* 2001;4:201-14.

61. Deng AY, Jackson CM, Hoebee B, Rapp JP. Mapping of rat Chromosome 2 markers generated from chromosome-sorted DNA. *Mammalian Genome* 1997;8:731-5.

62. Deng AY, Dene H, Rapp JP. Mapping of a Quantitative Trait Locus for Blood Pressure on Rat Chromosome 2. *Journal of Clinical Investigation* 1994;94:431-6.

63. Deng AY, Dene H, Rapp JP. Congenic Strains for the Blood Pressure Quantitative Trait Locus on Rat Chromosome 2. *Hypertension* 1997;30:199-202.

64. Kren V. Genetic Isolation of a Region of Chromosome 8 That Exerts Major Effects on Blood Pressure and Cardiac Mass in the Spontaneously Hypertensive Rat. *Journal of Clinical Investigation* 2004;99:577-81.

65. Kovacs P, Voigt B, Kloting I. Novel Quantitative Trait Loci for Blood Pressure and Related TRaits on Rat Chromosomes 1, 10 and 18. *Biochemical and Biophysical Research Communications* 1997;235:343-8.

66. Zagato L. Genetic Mapping of Blood Pressure Quantitative TRait Loci in Milan Hypertensivee Rats. *Hypertension* 2004;36:734-9.

67. Deng AY. Genetic Mapping of two new blood pressure quantitative trait loci in the rat by genotyping Endothelin System Genes. *Journal of Clinical Investigation* 2004;93:2701-9.

68. Cicila GT, Garrett MR, Lee SJ, Liu J, Dene H, Rapp JP. High-Resolution Mapping of the Blood Pressure QTL on Chromosome 7 using Dahl Rat Congenic Strains. *Genomics* 2001;72:51-60.

69. Rat Genome Sequencing Project Consortium. Genome sequencec of the Brown Norway rat yields insights into the mammilian evolution. *Nature* 2004;428:493-

70. Garret MR, Dene H, Walder R, Zhang Q, Cicila GT, Assadnia S, Deng AY and Rapp JP. Genomic scan and congenic strains for blood pressure quantitative trait loci using Dahl salt-sensitive rats. *Genome Research* 8: 711-723, 1998.

71. Garret MR and Rapp JP. Two closely linked interactive blood pressure QTL on rat chromosome 5 defined using congenic Dahl rats. *Physiological Genomics* 8: 81-86, 2001.

72. Pravenec M, Gauguier D, Schott JJ, Buard J, Kren V, Bila V, Szpirer C, Szpirer J, Wang JM, Huang H and . Mapping of quantitative trait loci for blood pressure and cardiac mass in the rat by genome scanning of recombinant inbred strains. *J Clin Invest* 96: 1973-1978, 1995.

73. Stoll M, Cowley AW, Jr., Tonellato PJ, Greene AS, Kaldunski ML, Roman RJ, Dumas P, Schork NJ, Wang Z and Jacob HJ. A genomic-systems biology map for cardiovascular function. *Science* 294: 1723-1726, 2001.

74.Kaneko, Y., Herrera, V. L., Didishvili, T., and Ruiz-Opazo, N. Sexe-specific effects of dual ET-1/ANG II receptor 9 (Dear) variants in Dahl salt-sensitive/resistant hypertension rat model. Physiol Genomics, *20*: 157-164, 2005.

75. Yagil, C., Sapojnikov, M., Kreutz, R., Katni, G., Lindpaintner, K., Ganten, D., and Yagil, Y. Salt susceptibility maps to chromosomes 1 and 17 with sex specificity in the Sabra rat model of hypertension. Hypertension, *31*: 119-124, 1998.

76. Mattson, D. L., Kunert, M. P., Kaldunski, M. L., Greene, A. S., Roman, R. J., Jacob, H. J., and Cowley, A. W., Jr. Influence of diet and genetics on hypertension and renal disease in Dahl salt-sensitive rats. Physiol Genomics, *16*: 194-203, 2004.

77. Hamet, P., Pausova, Z., Adarichev, V., Adaricheva, K., and Tremblay, J. Hypertension: genes and environment. J.Hypertens, *16*: 397-418, 1998.

78. Dutil, J. and Deng, A. Y. Further chromosomal mapping of a blood pressure QTL in Dahl rats on chromosome 2 using congenic strains. Physiological Genomics, *6*: 3-9, 2001.

79. Hamet, P., Pausova, Z., Adarichev, V., Adaricheva, K., and Tremblay, J. Hypertension: genes and environment. J.Hypertens, *16*: 397-418, 1998.

80. Brockway, B. P. and Hassler, C. R. Application of radiotelemetry to cardiovascular measurements in pharmacology and toxicology. *In* E. Salem and S. I. Baskin (eds.), New technologies and concepts for reducing drug toxicities, pp. 109-132. Boca Raton, Florida: CRC Press, 1993

81.Smirk, F. H. and Hall, W. H. Inherited hypertension in rats. Nature, *182*: 727-728, 1958.

82. Dahl, L. K., Hein, M., and Tassinari, L. Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt ingestion. Nature, *194*: 480-482, 1962.

83.Dahl, L. K., Hein, M., and Tassinari, L. Effects of chronic excess salt ingestion: evidence that genetic factors play an important role in susceptability to experimental hypertension. J.Exp.Med., *115*: 1173-1190, 1963.

84. Kato, N., Hyne, G., Bihoreau, M. T., Gauguier, D., Lathrop, G. M., and Rapp, J. P. Complete genome searches for quantitative trait loci controlling blood pressure and related traits in four segregating populations derived from Dahl hypertensive rats. Mamm.Genome, *10*: 259-265, 1999.

85. Deng, A. Y. Functional genomics of blood pressure determination: dissecting and assembling a polygenic strait by experimental genetics. Current Hypertension Reviews, *1*: 35-50, 2005.

86. Palijan, A., Lambert, R., Sivo, Z., Dutil, J., and Deng, A. Y. Comprehensive congenic coverage revealing multiple BP QTLs on Dahl rat Chromosome 10. Hypertension, *42*: 515-522, 2003.

87. Palijan, A., Dutil, J., and Deng, A. Y. Quantitative trait loci with opposing blood pressure effects demonstrating epistasis on Dahl rat chromosome 3. Physiological Genomics, *15*: 1-8, 2003.

88.Lander, E. S. and Schork, N. J. Genetic dissection of complex traits. Science, *265*: 2037-2048, 1994.